IonWorks Barracuda®

Automated Patch Clamp System

Hardware and Software

User Guide

MOLECULAR DEVICES

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ISO 9001 REGISTERED COMPANY

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Molecular Devices, LLC is ISO 9001 registered.

Contents

Chapter 1: Getting Started

This section provides information for getting started with the Automated Patch Clamp System. It is intended for new users. Before using the instrument for the first time, make sure you read the Safety Guide on page 193.

Daily Operation Overview

This daily operation overview provides a summary of the basic steps required to use the IonWorks Barracuda® instrument for a set of experiments. For specific instructions on performing each of these steps, see the relevant topic and follow instructions carefully.

Daily operation tasks include:

This section briefly describes each task.

Prepare the Instrument

Prepare the instrument by filling source bottles and emptying the waste carboys:

1. Turn the system on and start the IonWorks Barracuda® System Software. For instructions, see Starting Up the IonWorks Barracuda System on page 11.
2. Prepare the system fluids.

- Empty waste carboys in the system cabinet below the process deck.
- Fill source bottles.
  Each experiment run uses approximately 30 mL of external buffer, 50 mL of internal buffer, and 20 mL of cell perforation agent. Adjust the final volume based on the total number of intended experiment runs. Remember to add the dead-volume for each bottle: 15 mL for 250 mL bottles, 30 mL for 500 mL bottles, and 60 mL for 1000 mL bottles. Fill the alcohol (EtOH) bottle with a minimum volume of 100 mL for the Flush and Rinse.
- Fill wash station Source A and Source B carboys.

3. Using an undamaged PatchPlate and a CellPettor pipette, run **Start of Day Flush and Rinse** to initializes the system, eliminate alcohol (EtOH) from the system, and prime with saline solutions. For instructions, see Performing the Start of Day Flush and Rinse on page 105.
4. Load the required labware onto the process deck:

- Load a clean buffer boat at the external buffer station and fill it with 25 mL to 125 mL of external buffer.
- Load a new PatchPlate at the PatchPlate station.
- Load a clean cell boat at the cell boat station. If you are using the CellPettor, make sure you are using a Molecular Devices standard cell boat.
- If applicable, load the tip rack station with a tray of disposable pipettor tips.

For detailed instructions, see Preparing the Instrument for an Experiment Run on page 101.

Prepare Compounds and Cells

To prepare compounds and cells:

1. Prepare a compound plate and load it at the applicable compound station.
2. Prepare the cell suspension, resuspend the cells in a 15 mL conical tube, and load the tube into the cell tube holder. If you are not using the CellPettor, manually pipette the cell suspension to the cell boat.

For detailed instructions, see Loading Compound Plates on page 110 and Preparing Cell Suspension on page 111.

Select Protocols

There are three types of protocols for each experiment, and a saved protocol option needs to be selected for each type in order to run an experiment.

- **Setup Protocol**: Performs cell preparation steps including plate setup, vacuum checks, electrical checks, plenum filling, hole test, seal test, and perforation of the cell membrane. The purpose of this protocol is to prepare a new PatchPlate with cells before the Channel Protocol.
- **Channel Protocol**: Performs the compound addition, voltage application, and data acquisition steps in the experiment. The purpose of this protocol is to measure ionic currents from the cells in the PatchPlate.
- **Cleanup Protocol**: Performs wash and drain operations after an experiment completes. The purpose of this protocol is to ensure that all instrument components are clean and ready for the next experiment.

Run the Experiment

You can run experiments in the following modes:

- **Assay Development Mode:** During Assay Development Mode, the selected Channel Protocol runs and pauses. At the pause, you can adjust parameters to modify the Channel Protocol. The assay continues to pause after each iteration of the Channel Protocol until you have optimized for your desired result. You can then save the optimized Channel Protocol as a new protocol, which can be run later in Multiple Protocol mode or Single Protocol mode. See Modifying a Channel Protocol in Assay Development Mode on page 47. If you are running in Assay Development Mode, follow the prompts at the pause to run through iterations of the Channel Protocol, or complete the experiment by proceeding to the Cleanup Protocol.
- **Multiple Protocol Mode**: In Multiple Protocol mode, you can select multiple Channel Protocols to run without interruption, except for specified pauses in operations to add or change compound plates or to add cells. Experiments are conducted using one Setup Protocol, any number of Channel Protocols, and one Cleanup Protocol.
- **Single Protocol Mode**: In Single Protocol Mode, the selected protocols run without interruption. One Setup Protocol, one Channel Protocol, and one Cleanup Protocol run through to completion, except for specified pauses in operations to add or change compound plates or to add cells.

For detailed instructions, see Running an Experiment on page 117.

Analyze the Data

After the experiment runs, the acquired data can be analyzed.

To analyze experiment data:

1. Select the Data Analysis workspace.
2. Select the scan to review from the list of experiment steps.

- Zoom the well view to inspect results more closely.
- Apply metrics to reduce data for metric export.
- Apply filters to remove the less useful data from the results.
- Apply summary criteria to refine hit data.

For detailed instructions, see Analyzing Data on page 133.

Export Data

To export data:

1. Open the **Export Metrics** dialog.
2. Select the scans and User Metrics to export.
3. Specify the Metric File Name.
4. Select the File Format.
5. Click **Export**.

For detailed instructions, see Analyzing Data on page 133.

Clean up the Instrument

At the end of the day, clean up the instrument.

The most important step in cleaning up for the day is to run the **End of Day Flush and Rinse**because it pumps out saline solutions and sterilizes the equipment and source bottles with a 50% Ethyl Alcohol (EtOH) rinse and backfill.

To clean up the instrument:

1. After the experiment is completed, discard unused solutions and rinse the cell boat, and buffer boat with a 50% Ethyl Alcohol (EtOH) solution followed by DI water.

   Note: Leave the last PatchPlate in place to use for the next **Start of Day Flush and Rinse** run.

2. Unload the pipettor tips. You can do this manually using instrument utilities, or you can include this step in the Cleanup Protocol using the **Discard Tips** option. Skip this step if you are planning to re-use the tips.

   Note: Tips can be unloaded from the pipettor head and reloaded approximately nine times before you must replace the tip rack with new tips.

3. Empty all of the fluid bottles under the process deck, except the Ethyl Alcohol bottle. See The Fluid Handling Components on page 40.
4. Run **Utilities > End of Day Flush and Rinse**. See Performing the End of Day Flush and Rinse on page 149.
5. Unload the CellPettor pipette.

6. Empty the waste carboys. The waste carboys must be emptied daily, either at the end of the day or before the next day's operations. See Emptying Waste Carboys on page 102.
7. Check the overflow reservoir (sink) in the lower section of the instrument cabinet to the right of the source carboys. The overflow reservoir is a catch for any spills that might occur at the PatchPlate station or on the process deck. The capacity of the reservoir is limited, so if any fluid is present, sponge the liquid out to prevent overflow of the reservoir. If liquid is present, but you do not recall any spills, contact Molecular Devices Technical Support.

For more details, see Completing Instrument Cleanup on page 149.

Starting Up and Shutting Down

The IonWorks Barracuda® instrument starts up in combination with the IonWorks Barracuda® System Software. The instrument can be shut down independently so that you can continue to use the software offline to access data or edit protocol specifications. Multiple instances of the Analysis only software can run simultaneously, because it does not control the instrument, while one instance of the Barracuda data acquisition software runs controlling the instrument.For more information, see Working Offline in Analysis Only Mode on page 21.

The instrument might require initializing. During initialization, the software confirms that instrument components are properly calibrated and positioned, used fluids are pumped out, and new fluids are available for the next experiment.

Starting Up the IonWorks Barracuda System

The IonWorks Barracuda® System Software runs in two modes:

- **Barracuda**: Software controls the instrument for data acquisition, as described in this topic.
- **Analysis only**: Software does not control the instrument. For more information, see Working Offline in Analysis Only Mode on page 21.

Note: One instance of the online data acquisition software can run simultaneously with multiple instances of the Analysis only software running offline.

To start the IonWorks Barracuda System:

1. On the right-side power supply panel, turn on power. This primary power-switch turns on the instrument, the computer, and the monitor.
2. Log into the computer operating system.
3. Start the IonWorks Barracuda® System Software by double-clicking the **IonWorks Barracuda** icon.

Before running experiments:

1. Run **Calibrate CellPettor** from the **Utilities** dialog using a dry CellPettor Pipette. See Using Manual Instrument Controls on page 151.
2. Run **Start of Day Flush and Rinse** to pump out residual alcohol from the tubes and reservoirs, and prime the system with assay solutions. For instructions, see Performing the Start of Day Flush and Rinse on page 105.

Shutting Down the IonWorks Barracuda System

Before shutting down the instrument, run the End of Day Flush and Rinse procedure so that the instrument is in a sterilized state. For instructions, see Performing the End of Day Flush and Rinse on page 149.

To shut down the IonWorks Barracuda System after normal operations:

1. Click **Instrument > Disconnect from Instrument**.
2. Exit the IonWorks Barracuda® System Software by clicking **File > Exit**.
3. Turn off the computer by clicking **Start > Shut Down**.
4. On the right-side power supply panel, turn off power. This primary power-switch turns off the instrument, the computer, and the monitor.

Resetting the Instrument after an Emergency Stop

A red emergency-stop button is built into the front of the instrument cabinet. If an emergency occurs while the instrument is running, press the emergency-stop button to immediately cease all instrument functions.

To reset the instrument after an emergency stop:

1. Pull the red emergency-stop knob and twist it clockwise until it stops.
2. Click  , and then click **Initialize**. During initialization, the previous operation cancels and the instrument resets to the **Ready** state.
3. Click **Close**.

If errors occur preventing the instrument from initializing, shut down and restart the instrument. See Shutting Down the IonWorks Barracuda System on page 12, and then Starting Up the IonWorks Barracuda System on page 11.

Chapter 2: Software Overview

2

IonWorks Barracuda® System Software controls the functions of the instrument, displays acquired data, and provides data analysis and export functions after an assay completes. The software comes pre-installed on the system computer.

To install the software on additional computers in Analysis only mode, see Installing IonWorks Barracuda™ Software on page 22.

Two desktop icons are available to start the software:

Icon	Function
	Starts the software controlling the instrument.
	Starts the software in Analysis only mode (offline, not controlling the instrument). See Working Offline in Analysis Only Mode on page 21 for more information.

IonWorks Barracuda® System Software consists of three main workspaces:

- Data Acquisition and Review Workspace on page 15
- Data Analysis Workspace on page 16
- Protocol Editors on page 17

Optional Monitor Software:

IonWorks Barracuda Monitor Software is available for free to remotely view completed experiment data acquired on the IonWorks Barracuda System through an Internet connection. You can use it with a Web browser or an iPad. IonWorks Barracuda Monitor Software enables you to:

- Select data folders
- Select completed experiments
- See experiment status
- Read experiment notes
- View PatchPlate scans and summaries
- Read protocol names
- Review quality control data

To get the IonWorks Barracuda Monitor Software:

- For iPad monitoring, download from the Apple App Store.
- For web-browser monitoring, contact Molecular Devices Technical Support for the installer.

The Shortcut Bar

The shortcut bar is the name for the group of large buttons on the left side of the screen. The following shortcuts are available from any workspace:

Shortcut Button	Description
	Access the Data Acquisition and Review workspace.
	Access the Data Analysis workspace.
	View the Associated Protocols summaries.

Shortcut Button	Description
 Metrics Export	Open the Metrics Export dialog.
 Utilities	Open the Instrument Utilities dialog.
 Help	View the Application Help.

Data Acquisition and Review Workspace

When you start the IonWorks Barracuda® System Software, the Data Acquisition and Review workspace appears. The PatchPlate wells show data after an experiment begins, or a completed experiment is selected.

In the Data Acquisition and Review workspace you can:

- Define experiment names
- Define protocols
- Run experiments in various modes
- Review acquired data at an individual plate well level

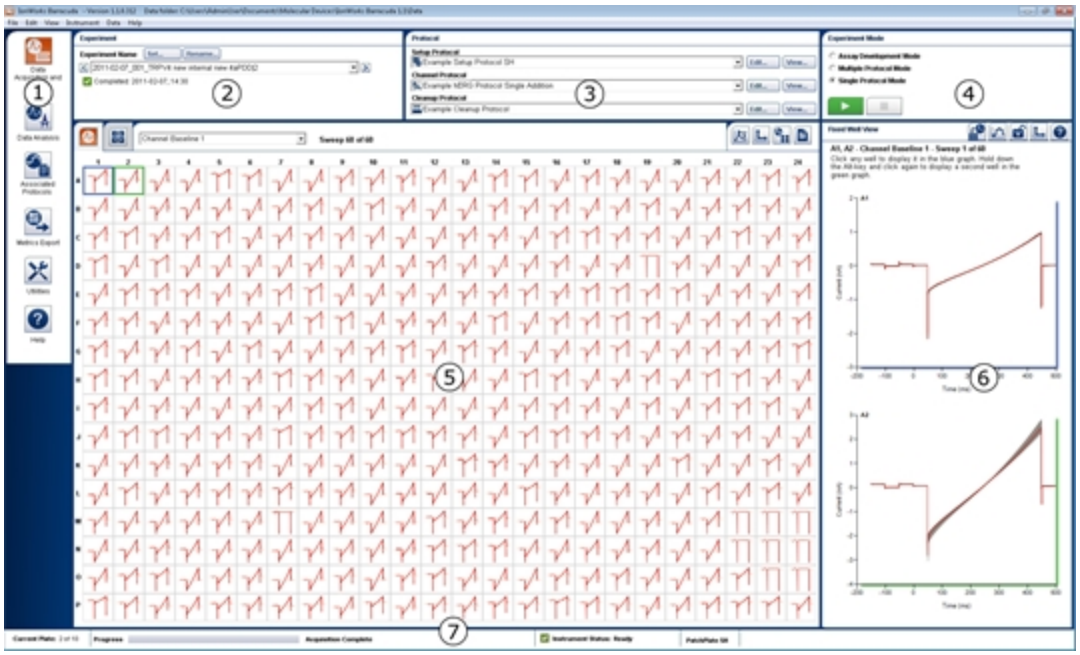


Figure 2-1: Data Acquisition and Review Workspace

Position	Component
1	**Shortcut Bar**: Open workspaces, view protocols associated with the data you are viewing, export data, or open online Help.
2	**Experiment**: Set the format for new experiment names, load a completed experiment, or rename a completed experiment.
3	**Protocol**: Select protocols or open protocol editors.
4	**Experiment Mode**: Select assay development mode, multiple protocol mode, or single protocol mode, and then start or stop an experiment run.
5	**Plate View**: Select trace view or extracted values view, select a scan to view, turn leak-correction on or off, or view experiment notes.
6	**Fixed Well View**: Select wells to zoom, view voltage waveform, or rescale trace graphs.
7	**Progress Bar** and **Instrument Status**: View the status of the selected experiment or view the status of the instrument.

Data Analysis Workspace

After data is acquired, you can refine your results in the Data Analysis workspace. To open the Data Analysis workspace, click **Data Analysis** from the shortcut bar on the left.

In the Data Analysis workspace you can:

- Define an unlimited number of single or combined metrics.
- Define filters to block out less useful data.
- Define summaries with threshold criteria to further refine hit data.

The Data Analysis workspace provides views that summarize responses and statistics based on the experiment type. In this workspace, you can perform basic analysis of acquired data using metrics, filters, and summaries.

Figure 2-2: Data Analysis Workspace

Position	Component
1	**Shortcut Bar**: Open workspaces, view protocols associated with the data you are viewing, export data, open Help.
2	**Experiment**: Select a completed experiment, set an experiment name.
3	**Plate Details**: View the results in a color-coded plate display based on filters and summaries.
4	**Color-key and Statistics**: View the color key and statistics for the plate display; statistics are based on defined summaries.
5	**Fixed Well View**: Select a well to zoom, view voltage waveform, rescale trace graphs.
6	**Filters and Summaries**: Edit native filters, create user-defined filters and summaries to refine hit results, access the Metric Editor, save current settings, and turn leak correction on or off.
7	**Well Statistics**: View metrics for individual wells.

Protocol Editors

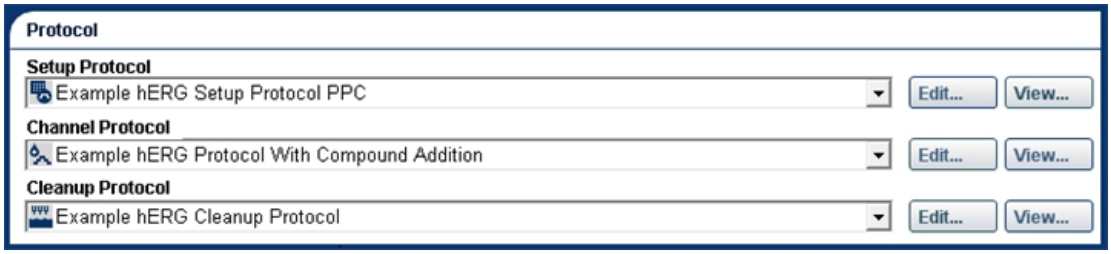
Every assay consists of a Setup Protocol, a Channel Protocol, and a Cleanup Protocol. Access the Protocol Editors by first selecting a protocol from the list of protocols in the Data Acquisition and Review workspace, and then clicking **Edit**.

When the editor appears, step through the tabs to modify each of the parameters for the protocol. Protocols can be saved with a new name and description, or saved as Read-Only. Channel Protocols include saved metrics settings that can be modified or imported for use with other Channel Protocols.

Using the protocol editors, you can:

- Create or Edit a Setup Protocol
- Create or Edit a Channel Protocol
- Create or Edit a Cleanup Protocol

Use the Protocol Editors to make changes to the parameters of any protocol. If the protocol has been marked read-only, give the modified protocol a new name and save it. Mark the new protocol as read-only if you plan to use it often or if you plan to run the experiment without interruption.



To open the Protocol Editors:

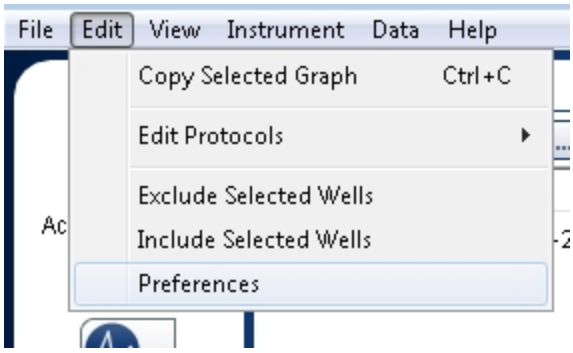
1. Select the name of an existing Setup, Channel, or Cleanup Protocol to start from by selecting the name of the protocol in the **Protocol** field.
2. Click the **Edit** button next to the protocol you want to edit. The Protocol Editor appears.

Setting Preferences

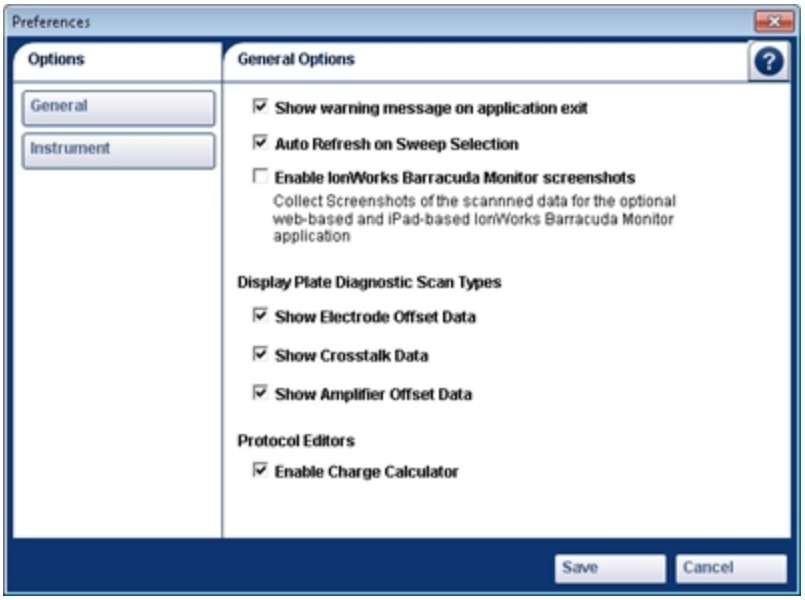
The Preferences dialog allows you to customize some general settings and some instrument settings. The general options involve the appearance of the software exit message, the plate diagnostic scans, and screenshots captured for use by the optional IonWorks Barracuda Monitor Software. The instrument options involve customizing parameters for the plenum vacuum, compound addition, and mixing.

To set preferences:

1. In the software menu, click **Edit > Preferences**.



The **Preferences** dialog appears and displays the **General Options** pane by default.

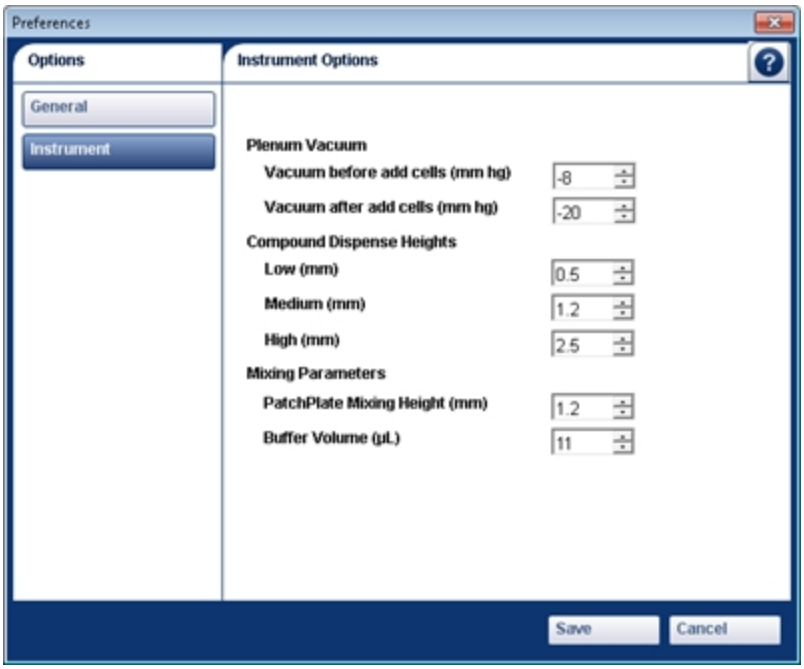


2. Clear or select a check box to hide, show, or disable available options. **General Options** include:

Option	Description
Show warning message on application exit	The message that appears by default before the software exits is hidden or shown.
Auto Refresh on Sweep Selection	The same sweep in the Plate view and the Fixed Well View displays automatically.
Enable IonWorks Barracuda Monitor screenshots	The scan data screenshots collected with the experiement data are captured or skipped. Impacts what can and cannot be reviewed in the Web-based, and iPad-based IonWorks Barracuda Monitor Software.
Display Plate Diagnostic Scan Types	The diagnostic scan types that appear in the plate view scans list is hidden or shown. Diagnostic scan types include: Electrode Offset, Crosstalk, and Amplifier Offset.
Protocol Editors	The **Enable Charge Calculator** check box enables the **Coulomb Meter** and **Estimated Seal Resistance** field in the **Edit Setup Protocol** dialog and the **Edit Channel Protocol** dialog.

Note: Showing and hiding options does not impact the acquisition of the data.

3. To view **Instrument Options**, click **Instrument** in the left pane, or from the software menu, click **Edit > Preferences > Instrument**.



4. Change the value of any of the available options. **Instrument Options** include:

Option	Setting	Value Range
Plenum Vacuum	Vacuum before add cells (mm hg)	Minimum: -40 Maximum: -8
	Vacuum after add cells (mm hg)	Minimum: -40 Maximum: -8
Compound Dispense Heights	Low (mm)	Minimum: 0.2 Maximum: 1.2
	Medium (mm)	Minimum: 0.5 Maximum: 2.5
	High (mm)	Minimum: 1.2 Maximum: 4.0
Mixing Parameters	PatchPlate Mixing Height (mm)	Minimum: 0.5 Maximum: 3.5
	Buffer volume (µL)	Minimum: 10 Maximum: 18

5. Click **Save**.

Working Offline in Analysis Only Mode

IonWorks Barracuda® System Software runs in two modes:

- **Analysis only**: Software does not control the instrument, for data analysis only, as described in this topic.
- **Barracuda**: Software controls the instrument, for data acquisition. For more information, see Starting Up the IonWorks Barracuda System on page 11.

Note: Multiple instances of the Analysis only software can run simultaneously, because it does not control the instrument, while one instance of the Barracuda data acquisition software runs controlling the instrument.

Two desktop icons are available to start the software:

To open the software in Analysis only mode, double-click the icon labeled **IonWorks [Analysis Only]**.

While using the software in Analysis only mode, you can:

- Create and edit protocols (but not run them)
- Define new metrics
- Analyze acquired data using metrics, filters, and summaries
- Create export methods and export data files

Disconnecting Control of the Instrument

If you are finished running assays for the day and have completed instrument cleanup, you can disconnect the control of the instrument to work in Analysis only mode.

To disconnect the software control of the instrument:

1. In the software menu, click **Instrument > Disconnect from Instrument**.
2. Click **Yes** at the prompt to confirm.

Reconnecting to the Instrument

If you disconnected the software control of the instrument to work in Analysis only mode, you can reconnect.

To reconnect the software control of the instrument:

1. Save any files you are working on.
2. Exit the software.
3. Restart the software by double-clicking the **IonWorks Barracuda** icon.

Installing IonWorks Barracuda™ Software

IonWorks Barracuda® System Software can be installed on additional computers to work in Analysis only mode. Before installing the software, make sure the computer meets the following minimum system requirements:

- OS: Windows 7 (64-bit) and Windows 10 (64-bit)
- CPU: Quad core 2.4 GHz
- RAM: 8 GB
- Graphics card supporting a resolution of 1920 x 1200

Note: The software is designed for a 24-inch monitor with a display resolution of 1920 x 1200, and default display font setting. If you are installing on a computer with a lower resolution or smaller screen size, or change the computer font size to larger than the **100%** setting, you might experience minor display issues.

To install the software:

1. Insert the Automated Patch Clamp System CD into the drive. The installation process begins automatically.

   If you have disabled auto-play features, you can begin the installation by browsing the CD to locate the installer file: **BarracudaInstaller.exe**. Double-click the **BarracudaInstaller.exe** file to begin the installation process.
2. Follow the prompts to install the software. Make sure you choose the option for **Simulation Mode** when installing on any computer not connected to the instrument.

Chapter 3: Instrument Overview

This section provides a functional overview of the IonWorks Barracuda®instrument components. For information about the software, see Software Overview on page 13.

The major components of the IonWorks Barracuda System include:

The Instrument Cabinet

The instrument cabinet houses the components of the IonWorks Barracuda System. For dimensions and operational specifications, see Instrument Specifications on page 191.

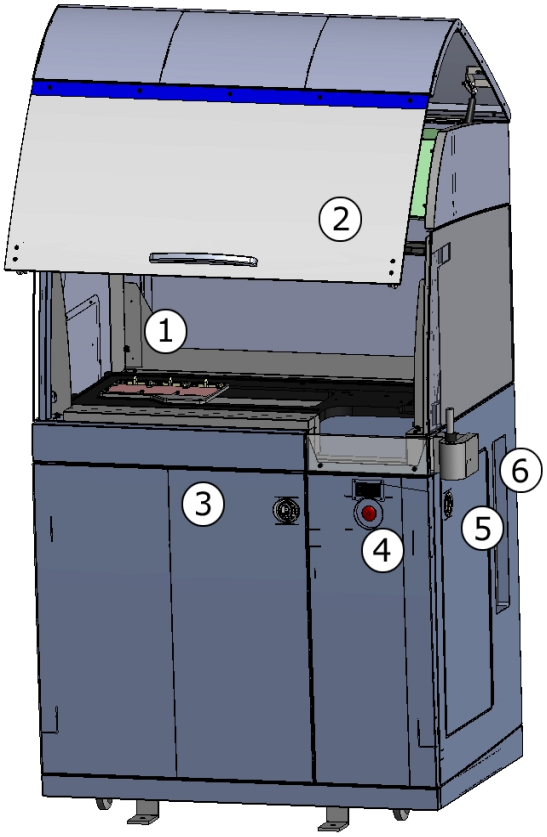


Figure 3-1: The IonWorks Barracuda System Instrument Cabinet

Position	Component
1	Upper compartment (Process deck)
2	Glass door
3	Lower compartment (Fluidics)
4	Emergency stop button
5	Right-side compartment
6	Power supply panel

Upper Compartment

The Process Deck, Pipettors, and Cell Handling components reside in the upper, glass-front compartment of the system cabinet. The upper compartment locks during automated operations.

Lower Compartment

The lower cabinet compartment encloses the fluid-handling system and the Patch Engine. The fluid-handling system includes fluid lines, pumps, valves, and sensors, plus source and waste carboys. The Patch Engine assembly clamps the Electrode-Plate to the plenum and controls vacuum pressure between the PatchPlate and the plenum nest during assays. The lower compartment locks during automated operations.

Emergency Stop Button

A red emergency-stop button is built into the front of the instrument cabinet. If an emergency occurs while the instrument is running, press the emergency-stop button to immediately cease all instrument functions.

Right-side Compartment

The right-side of the cabinet includes an enclosed fluid-handling area for the wash station and CellPettor with peristaltic pumps, a syringe pump, tubing, sensors, and a temporary waste bottle.

Power Supply Panel

The power supply panel is on the right side of the cabinet and includes:

- Voltage output (BNC) cable port
- Ethernet (CAT 5) port
- Covered exhaust fan outlet
- Four indicator lights:

Label	Indicates...
POWER	120-240 VAC (Universal Power Supply) power to the system computer
+24 VDC	24-volt DC power to components that run continuously
+24 VDC	24-volt DC power to interlocked components that switch off during access to the process deck
+48 VDC	48-volt DC power to interlocked components that switch off during access to the process deck

- System power switch
- System power cord outlet

The Process Deck

There are seven stations on the process deck. From left to right, the process deck stations include:

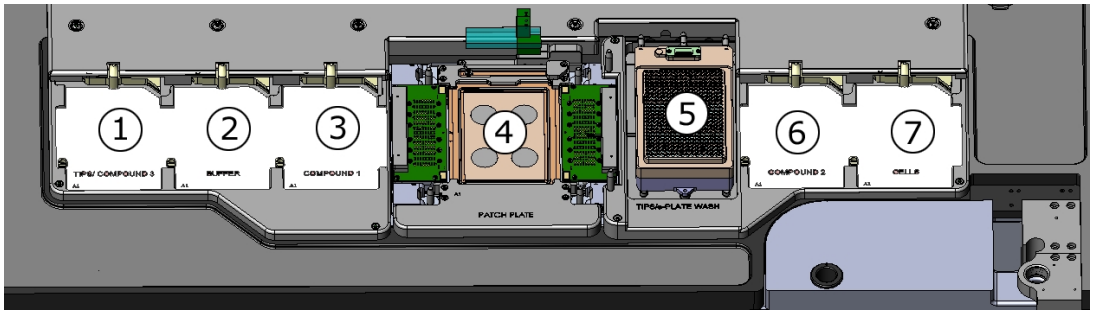


Figure 3-2: The Process Deck

Position	Station
1	Tip Rack (Do not use for compound plates)
2	External Buffer Boat
3	Compound 1
4	PatchPlate/Plenum
5	Wash Station
6	Compound 2
7	Cell Boat

Each of the stations is configured with an SBS-standard, 384-well microplate footprint to accommodate the 384-pipettor head. All assay steps take place on the process deck. The robotic pipettor head delivers fluids from the buffer boat, cell boat, and compound stations to the PatchPlate.

The Tip Rack Station

The tip rack station holds a Molecular Devices tray preloaded with 384 disposable micropipettor tips. Molecular Devices black tips [PN 5011976] or clear tips [PN 5011975] are approved for use with the Barracuda instrument. Black tips are recommended.

Note: Tips can be unloaded from the pipettors and reloaded approximately nine times before you must replace the tip rack with new tips.

The robotic pipettor head lowers onto the pipettor tip rack to load the disposable pipettor tips at the start of an experiment. The pipettor tips are used to aspirate and dispense external buffer solution, compounds, and cells within the PatchPlate as required throughout the duration of a protocol. The tips can be automatically washed for reuse as specified in Creating a Cleanup Protocol on page 97, or manually in the dialog.

Note: The tip rack station is engraved TIPS/COMPOUND 3. Please see specific instructions for adding three or more compounds since this position is not currently active for compound additions.

The External Buffer Station

The external buffer station holds an SBS-standard microplate with a 384-well footprint. Fill the external buffer boat with a minimum volume of 25 mL up to a maximum of 125 mL with your external buffer solution of choice prior to the start of any experiment. When filling the reservoir, make sure you dispense adequate total volume for each of the steps in your protocol.

CAUTION! Fill the boat or plate with adequate volume. Inadequate volume can cause the pipettor head to aspirate an air and fluid mixture that can ruin an experiment by dispensing air into the PatchPlate wells.

The Compound Stations

The Compound 1 and Compound 2 stations accommodate the SBS-standard footprint microplates listed in the Compound Addition section of the software. The pipettors aspirate compound from the Compound 1 or Compound 2 position and deliver it to the PatchPlate based on the protocol specifications.

Make sure you calculate appropriate total compound volume depending on the volume of each addition specified in the experiment protocol instructions.

Detailed volume specifications for allowed compound plates are available in the Compound Addition area of the Channel Protocol Editor. Click … next to the Plate Type list to display detailed volume specifications based on your plate type selection.

CAUTION! Fill the boat or plate with adequate volume. Inadequate volume can cause the pipettor head to aspirate an air and fluid mixture that can ruin an experiment by dispensing air into the PatchPlate wells.

The PatchPlate Station

The PatchPlate station consists of the plenum at its base, the consumable PatchPlate which rests in a nest in the plenum, and the Electrode-Plate which mechanically clamps to the assembly. The instrument creates a vacuum-seal between the PatchPlate and the plenum during assays. All experimental operations occur in the wells of the PatchPlate including fluid pipetting, cell dispensing, compound additions, and ion-channel recording.

The Wash Station

The Wash station is a fixed reservoir on the process deck designed to accommodate the pipettor tips and the Electrode-Plate electrode pins for washing procedures. Solutions for wash operations are pumped through the Wash station by the fluid-handling system and then emptied into the waste carboys below. Sensors monitor fluid levels to prevent overflow in the Wash station. An ultrasonic transducer provides an option for sonicating to thoroughly clean the Electrode-Plate electrode pins or to remove sticky compounds from the pipettor tips. The Wash station also serves as the home position for the Electrode-Plate.

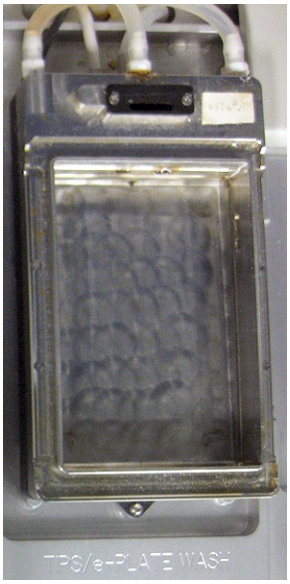


Figure 3-3: Wash Station

Wash station options include:

- The use of one or two solutions or solvents (Source A or Source B)
- The number of strokes per wash cycle (1 to 20)
- The total number of wash cycles (1 to 5)
- The option for ultrasonic cleaning

WARNING! Do not enable ultrasonic cleaning while using any flammable liquid such as alcohol (EtOH) in the Source A or Source B bottles. Sonication can cause flammable liquids to evaporate into the enclosure, posing a potential risk of fire or explosion.

CAUTION! Do not use the ultrasonic cleaning option when the wash station reservoir is empty. Dry sonication can lead to equipment failure.

The Cell Boat Station

The cell boat holds cells suspended in external buffer before they are aspirated by the pipettors and delivered to the PatchPlate. The cell boat station requires the use of approved cell boats only. Molecular Devices provides a custom-configured cell boat for use with the CellPettor that conserves cell suspension by reducing required cell boat volumes to approximately 5 mL [Molecular Devices PN 5005252].

Contact Molecular Devices Technical Support for information about adding an additional cell boat type to the IonWorks Barracuda® System Software list.

WARNING! Always wear protective equipment and review all applicable laboratory site regulations when handling cell solutions and cell waste. Always follow the appropriate procedures for the biosafety level of the substance you are handling.

CAUTION! The CellPettor cell-handling subsystem requires the use of Molecular Devices approved cell boats [Standard cell boat: Molecular Devices PN 5005252]. Using labware other than the Molecular Devices approved cell boats could damage the CellPettor.

The Patch Engine

The Patch Engine is the component of the IonWorks Barracuda System that digitizes data acquired during the automated patch clamp operation. The Patch Engine resides in the lower cabinet behind the internal buffer and cell perforation agent bottles, and beneath the PatchPlate Station.

The core components of the Patch Engine assembly are:

- **The Plenum**: The liquid-filled base of the Patch Engine that includes the ground electrodes and forms a vacuum beneath the PatchPlate.
- **The Electrode-Plate**: An array of 384 Ag/AgCl electrodes housed in a frame that fits on top of the PatchPlate and the plenum.
- **The Data Acquisition Engine**: The system that collects signals during assays. Each of the 384 electrodes on the Electrode-Plate connects to a single amplifier and digitizer to simultaneously convert the 384-channel recordings into usable data. This connection is made through the 384 pogo pins on the Electrode-Plate.

The Patch Engine assembly clamps the Electrode-Plate to the plenum maintaining a vacuum-tight seal between the PatchPlate and the plenum nest during assays.

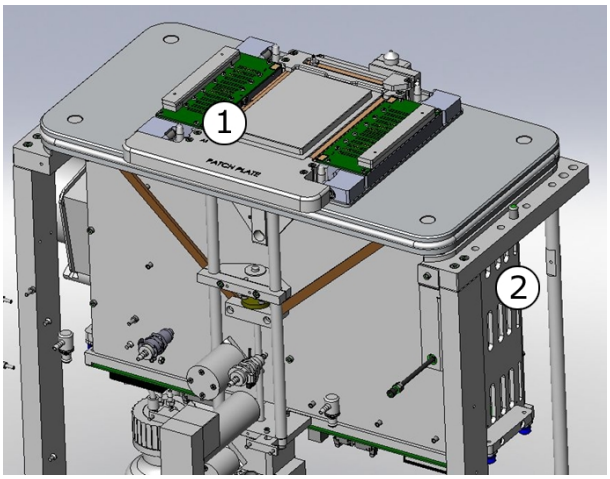


Figure 3-4: The Patch Engine

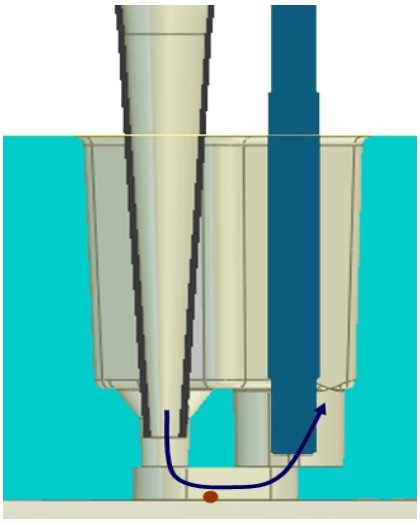
Position	Component
1	Amplifier touch pads
2	Amplifier boards beneath the plenum

PatchPlates

The consumable PatchPlate is a 384-well, SBS-standard footprint, planar patch microplate with a polyimide membrane at the bottom.

The capacity of each PatchPlate well is ~60 µL. A funnel guide in the PatchPlate wells ensures consistent positioning of the pipettor tips. The electrodes of the Electrode-Plate remain immersed in the PatchPlate wells for continuous recordings during assays. Fluid level is maintained in the wells between fluid additions by removing fluid from the wells after completing each compound addition and recording.

Note: PatchPlates are designed for a single use. Reuse PatchPlates for the Start of Day and End of Day Flush and Rinse procedures only.



Each well of the PatchPlate consumable features a flow-through design for rapid solution exchange enabled by:

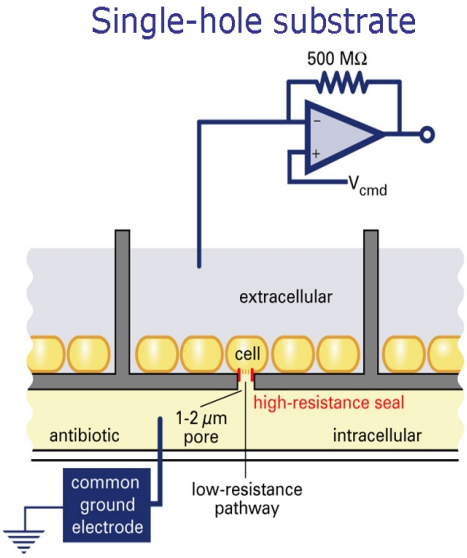
- A funnel guide to locate the pipettor tip to a consistent position within the well.
- User-defined dispense speeds and heights to control flow.
- Rapid individual offset correction for each of the 384 electrodes.

WARNING! Always wear protective equipment and review all applicable laboratory site regulations when handling cell solutions and cell waste. Always follow the appropriate procedures for the biosafety level of the substance you are handling.

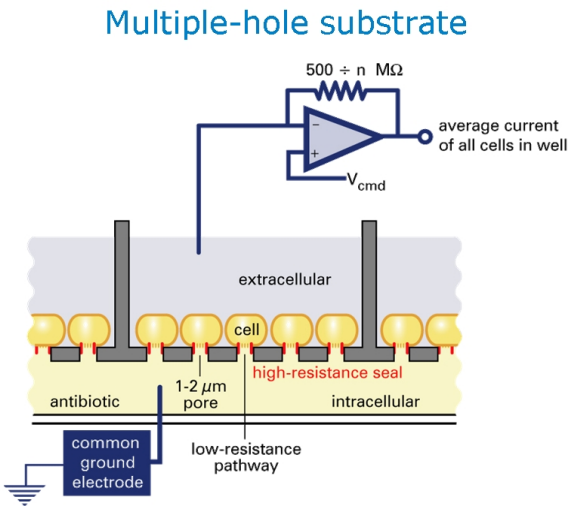
PatchPlate Types

There are several types of PatchPlates:

Single-Hole (SH) MegaSeal plates [Molecular Devices PN 5008697] contain a single ~2 µm hole in each well. Orange packaging labels designate SH plates.



Population Patch Clamp (PPC) MegaSeal plates [Molecular Devices PN 5008680] contain an array of 64 holes (~2 µm) in each well. Blue packaging labels designate PPC plates.



PatchPlate Well Configurations

The 384 wells of the PatchPlate are arranged in a grid of 16 rows by 24 columns. Each well in the PatchPlate is identified by a unique address. Only the **A1** well is physically labeled on the PatchPlate:

- The 16 rows are identified in the software as A–P.
- The 24 columns are identified in the software as 1–24.

Individual wells are identified by a row letter and a column number. When the PatchPlate is loaded onto the process deck, the well closest to the left-front of the system cabinet is **A1**. The right-rear well in this position is **P24**, the 384th well. All stations except the wash station are engraved with **A1** in the front-left position as a guide to properly loading plates.

PatchPlate Packaging, Storage, and Handling

The Molecular Devices consumable PatchPlate packaging, storage, and handling specifications include the following:

MegaSeal PatchPlates

- Each box contains 48 PatchPlates (12 packages of 4 plates each).
- Must be stored in a cool, dry place.
- SH packages labels are orange.
- PPC package labels are blue.
- Performance is assured for six months from the date of receipt.

Plenum

The fixed foundation of the PatchPlate station is called the plenum. The plenum enables delivery, from below, of internal buffer solution and cell perforation agent solution. The top surface of the plenum contains four ground electrodes.

CAUTION! Do not touch the round, ground electrodes on the surface of the plenum. Doing so could result in a build-up of oils on the electrodes. Oil buildup on the electrodes impedes their functionality.

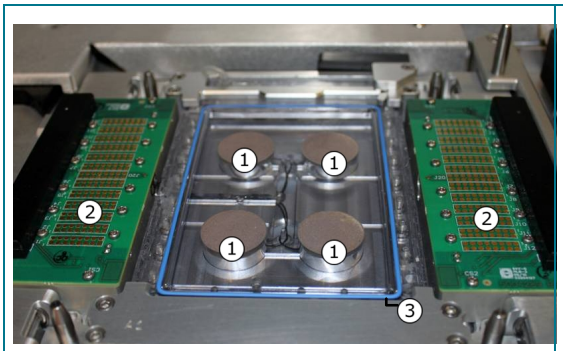


Figure 3-5: The Standard Plenum

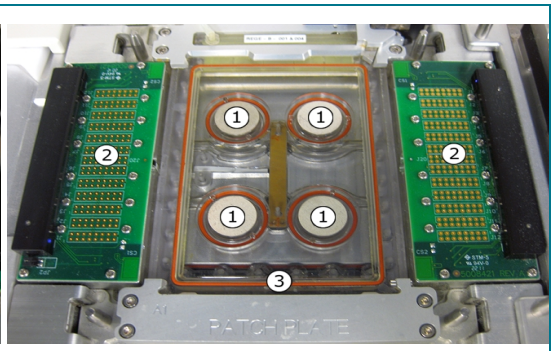


Figure 3-6: The Replaceable Ground Electrodes (REGEs) Plenum

Position	Component
1	Ground electrodes.
2	Amplifier touch pads.
3	Plenum O-ring (rubber gasket). The color of the O-ring can vary.

The PatchPlate and the Electrode-Plate are clamped onto the plenum to seal fluids during an assay. Source solutions are pumped into, and waste fluids are pumped out of, the plenum throughout an experiment. An O-ring surrounding the perimeter of the plenum permits the creation of a seal with the PatchPlate. A small negative pressure is used to pull the cells into the holes of the PatchPlate.

CAUTION! Always keep the plenum dry. Before loading a new PatchPlate, avoid the cable on the left side of the plenum and gently blot the plenum with a lint-free cloth.

A pressure sensor on the fluidics system monitors the PatchPlate-plenum seal and reports the vacuum pressure.

To check the plenum vacuum pressure:

- In the software, click **Instrument > View Plenum Vacuum Pressure**.

There are two styles of plenum, the standard plenum incorporating fixed ground electrodes, and the replaceable ground electrodes (REGEs) version. Your instrument will have one or the other style installed. To upgrade from a standard plenum to a REGE plenum, contact your Molecular Devices representative.

In addition to including four removable ground electrodes, the REGE plenum includes multiple protective features:

- Double O-rings in each plenum socket guard against internal buffer entering the plenum socket.
- Hermetically sealed plenum sockets assure that if the double O-ring fails, liquid stays in the plenum.
- Silver plated plenum socket metals prevent adverse junction potentials.

Replaceable Ground Electrodes (REGEs)

IonWorks Barracuda instruments are in the process of having a plenum upgrade installed with four replaceable ground electrodes (REGEs). See Plenum on page 34.

IonWorks Barracuda instruments using the REGE Plenum, come with a second set of four REGEs, four spare large plenum socket O-rings, four spare socket springs, and a service tool. A REGE Kit is also available if you want to purchase additional REGE sets. See Accessories on page 189.

Each REGE is encased in a serialized plastic housing that incorporates screw threads for installation and removal, and two small holes in the housing on opposite sides of each other to accommodate the custom service tool.

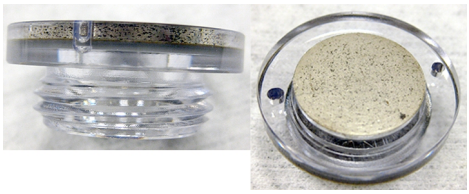


Figure 3-7: Side and Top View of a Replaceable Ground Electrode

Each REGE set is serialized. On the bottom of each housing in a set is a serial number and a letter. Each set should have the same serial number along with the letter A, B, C, or D to track each REGE location within the plenum slots, as needed. Molecular Devices recommends using the REGEs as complete sets. Avoid mixing multiple sets.

Figure 3-8: Serialized Set of Replaceable Ground Electrodes

For instrument maintenance purposes, Molecular Devices recommends removing and conditioning each installed REGE set nightly. See Conditioning Replaceable Ground Electrodes (REGEs) on page 153.

Electrode-Plate

The Electrode-Plate consists of an array of 384 silver chloride-coated electrodes housed in a frame which fits on top of the PatchPlate and the plenum. When the Electrode-Plate is clamped to the plenum, two banks of spring-loaded pogo-pins outside of the electrode array connect with the 384 amplifiers residing beneath the process deck. Holes in the Electrode-Plate provide access for the pipettor tips so that ion-current recordings are possible during the simultaneous addition of compounds to the PatchPlate wells.

Two types of Electrode-Plate are provided with your instrument, one Standard Electrode-Plate with green printed circuit boards, and one RA1 Electrode-Plate with black printed circuit boards. The RA1 Electrode-Plate uses pins that have a reduced silver chloride surface area compared to the pins in the Standard Electrode-Plate.

Stable recording of ion channel currents is crucial for pharmacological analysis of drug candidates. With other conditions being the same, the RA1 Electrode-Plate provides a significantly prolonged assay window for Nav1.5 channels. Improvements can also be seen on other channel types as well. Molecular Devices recommends that you try both Electrode-Plate to determine which one is best for your specific assay. Track which Electrode-Plate is used in your protocol in the **Experiment Notes** dialog.

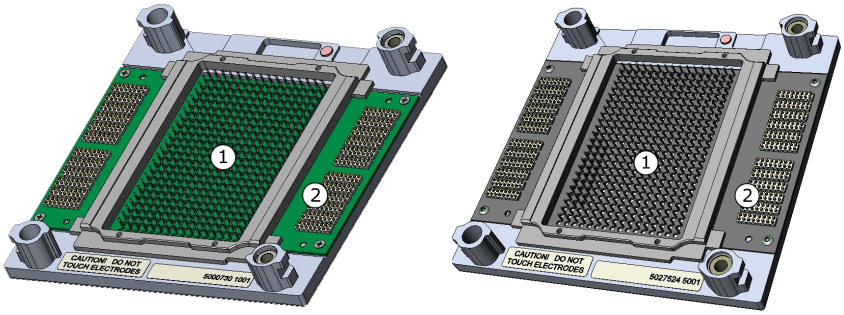


Figure 3-9: The Standard Electrode-Plate and the RA1 Electrode-Plate

Position	Component
1	Electrodes (measure current in the wells)
2	Pogo pins (connect to amplifiers)

After a PatchPlate has been loaded and the assay begins, the pipettor transports the Electrode-Plate from its resting position at the wash station to the PatchPlate station.

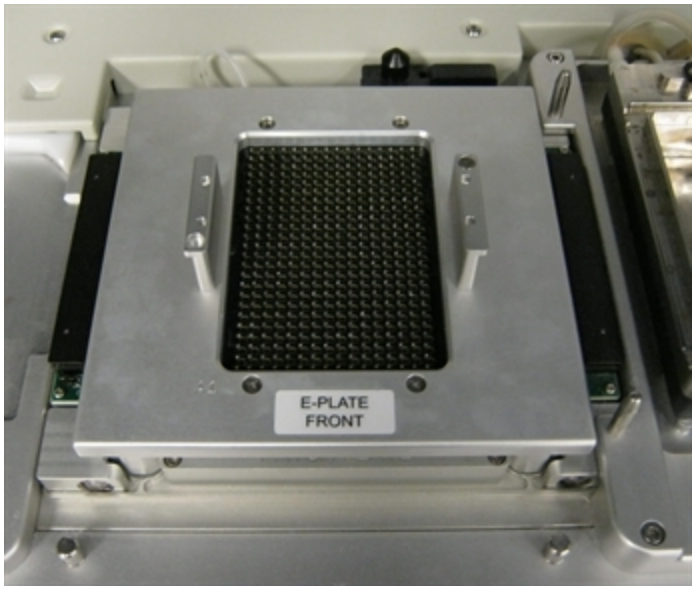


Figure 3-10: RA1 Electrode-Plate Clamped Onto the Plenum

The Electrode-Plate can deliver voltages in a range from -200 mV to +200 mV depending on the voltages specified in the software protocols.

CAUTION! To avoid damage to the Electrode-Plate:

- Do not touch the electrode pins or the pogo pins on the underside of the Electrode-Plate.
- Keep the pogo pins dry.
- Never immerse the Electrode-Plate in liquid of any kind. The electrode pins, however, can be immersed in a bleach solution for rechloriding.
- Always lift the Electrode-Plate using the handles on the top.
- Always set the Electrode-Plate on a flat surface resting on its four stand-offs or on the Bleach Boat.

The Pipettors

The head of the pipettor robot holds 384 distinct micropipettors that operate simultaneously to transport fluids from the cell boat, the buffer boat, and the compound plates to the PatchPlate wells. The pipettors aspirate and dispense fluids in precise amounts according to instructions specified in the software protocols. If the protocol set up in the software specifies it, the pipettors can circulate solutions within the wells by aspirating and dispensing small amounts.

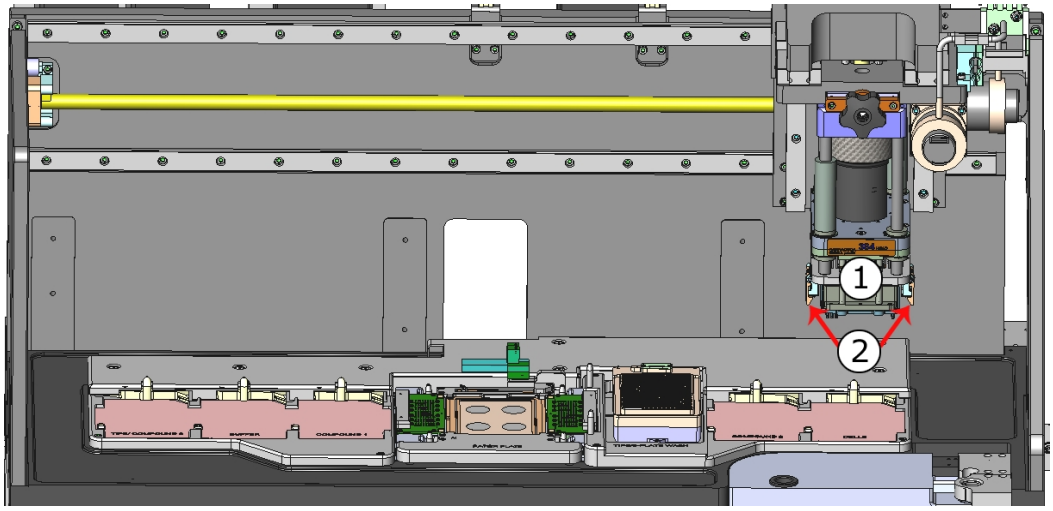


Figure 3-11: The Pipettors

Position	Component
1	Pipettor head (pipettors)
2	Electrode-Plate transport clips

Pipettor Tips

At the start of an experiment run, the system loads disposable tips onto the pipettor head from the tip rack. The disposable pipettor tips can be washed between runs for reuse as specified in the Cleanup Protocol. For details, see Creating a Cleanup Protocol on page 97.

Molecular Devices black tips [PN 5011976] or clear tips [PN 5011975] are approved for use with the Barracuda instrument. Black tips are recommended.

Electrode-Plate Transport

The pipettor head also serves as a transport mechanism for the Electrode-Plate, moving it from its resting position at the wash station to the PatchPlate station during assays, and then back to the wash station upon completion.

The Cell Handling Components

The IonWorks Barracuda System includes an automated cell-handling unit called the CellPettor, meaning "cell pipettor." The CellPettor delivers cell suspension to the cell boat during experiments. You can also deliver cells manually to the cell boat by turning off the CellPettor. See Adding Cells on page 59.

The cell-handling components include:

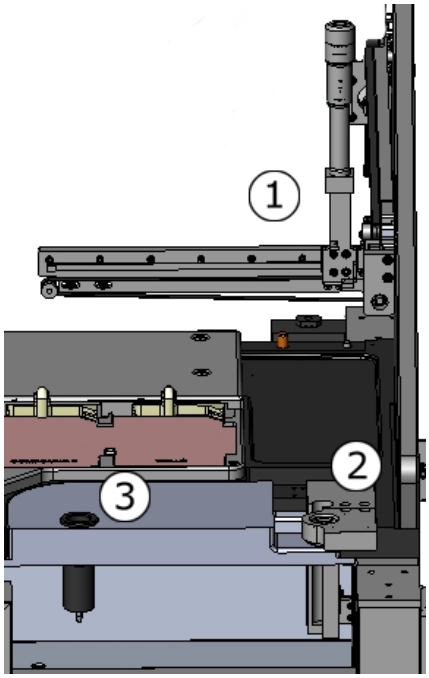


Figure 3-12: The Cell Handling Components

Position	Component
1	CellPettor
2	Cell tube holder
3	Cell waste sink

The CellPettor

The CellPettor is a robotic pipetting unit positioned above the process deck and near the cell boat station. The robot requires a standard 5 mL aspirating pipette with the cotton plug removed from the end, and a standard cell boat [Molecular Devices PN 500525] to operate.

The dimensions for the aspirating pipette should be:

- 8 mm diameter x 350 mm length

The Cell Tube

After you have prepared the cell suspension, pipette to a 15 mL conical tube for use with the CellPettor. Fill the cell tube with a minimum of 5 mL and a maximum of 10 mL of cell suspension. Load the conical tube of cell suspension into the cell tube holder in the frame of the process deck. The CellPettor aspirates prepared cell suspension from the conical tube and delivers the suspension in a serpentine pattern to the channels in the cell boat.

The dimensions for the cell tube should be:

- 17 mm diameter x 120 mm height

The Cell Waste Sink

Excess cell suspension is dispensed from the CellPettor to the 15 mL conical tube. The CellPettor pipette is washed with external buffer that flows to the cell waste sink. Finally, the sink fluids are pumped to the cell waste carboy below the process deck.

Note: Do not use the cell waste sink for any other waste.

CAUTION! The CellPettor cell-handling subsystem requires the use of Molecular Devices approved cell boats [Standard cell boat: Molecular Devices PN 5005252]. Using labware other than the Molecular Devices approved cell boats could damage the CellPettor.

The Fluid Handling Components

Using a network of tubing, pumps, valves, and sensors, the fluid-handling system delivers source fluids and pumps out waste fluids. Source bottles and waste bottles reside on holding racks below the process deck. Source bottles hold wash solutions and solvents, buffer solutions, perforation agent, and alcohol (EtOH). Waste carboys hold expelled solutions and cell waste.

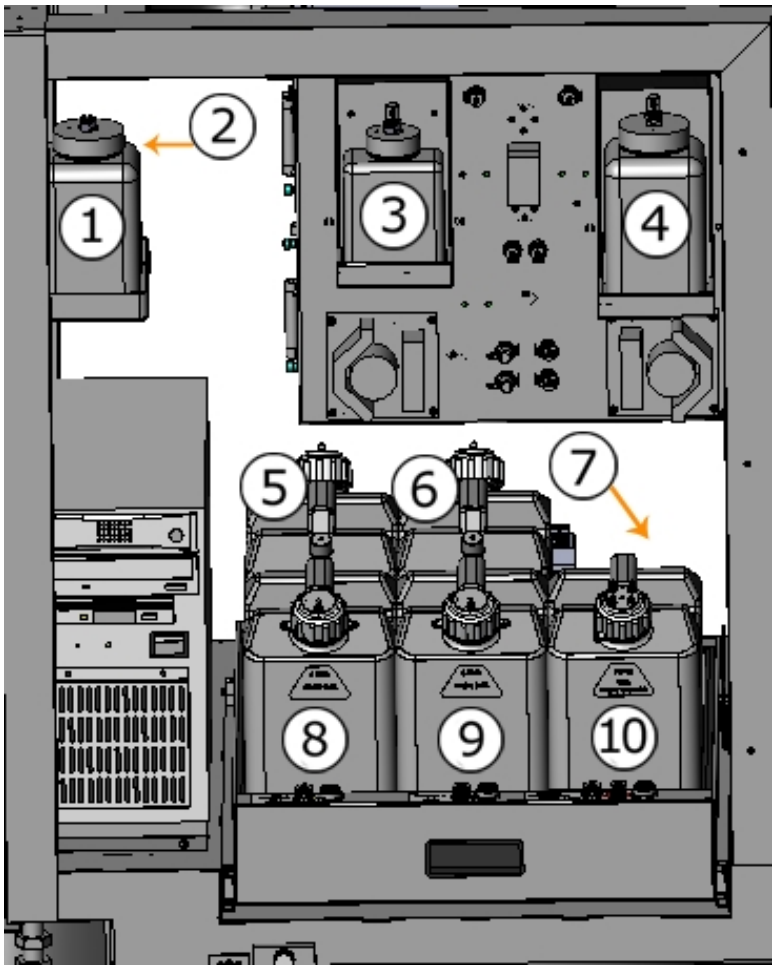


Figure 3-13: The Fluid Handling Components

Position	Component
1	Alcohol (EtOH)
2	External buffer solution (behind the alcohol source bottle)
3	Cell perforation agent
4	Internal buffer solution
5	Source A (wash solution)
6	Source B (wash solution)
7	Overflow reservoir
8	Waste A
9	Waste B
10	Plenum/Cell Waste

Source Fluid Bottles

The upper holding racks accommodate external buffer, alcohol, Amphotericin (or other cell perforating agent), and internal buffer bottles. These fluids must be filled with a minimum working volume of 100 mL before beginning the Start of Day Flush and Rinse procedure or a series of experiments.

Source A and B Wash Solution Carboys

Two six-liter bottles of wash solution are housed in the lower fluidics drawer at the rear of the system cabinet (Source A and Source B). Source bottles must be filled prior to the start of an experiment run and placed in the correct positions in the fluidics drawers. See Preparing the Fluid-Handling System on page 101.

Waste A and B Carboys

Two six-liter waste bottles are housed in the fluidics drawer in the front of the system cabinet (Waste A and Waste B). Always empty them at the beginning of the day, or prior to the start of an experiment run. Although the waste bottles include sensors to prevent overflow, overflow damages the vacuum pump so make sure you empty waste carboys regularly. The vacuum pump drains fluid waste from the wash station and delivers the waste fluids first to a temporary waste bottle, and finally to the Waste A and Waste B carboys. See Emptying Waste Carboys on page 102.

Plenum and Cell Waste Carboy

A third six-liter bottle for (Plenum/Cell Waste) holds cell-contaminated fluids collected from the cell handling system and the plenum including cell suspension, internal buffer, and perforation agent. The Plenum/Cell Waste bottle must also be emptied prior to the start of an experiment run to prevent overflow. See Emptying Waste Carboys on page 102.

Overflow Reservoir

A low-capacity overflow sink, or open reservoir, is located behind the plenum/cell waste carboy. The reservoir captures any spill or overflow that might occur on the process deck. The overflow reservoir should be checked periodically for liquid, and should be sponged out to prevent the reservoir from overflowing onto the floor. If the overflow reservoir has fluid in it, and you have not spilled any liquid, contact Molecular Devices Technical Support.

WARNING! Always wear protective equipment and review all applicable laboratory site regulations when handling cell solutions and cell waste. Always follow the appropriate procedures for the biosafety level of the substance you are handling.

The System Computer

The system computer resides inside the instrument cabinet on the lower left side. The system computer runs the pre-installed IonWorks Barracuda® System Software that controls automated instrument operations. The system computer must be in good working condition for the IonWorks Barracuda System to operate properly.

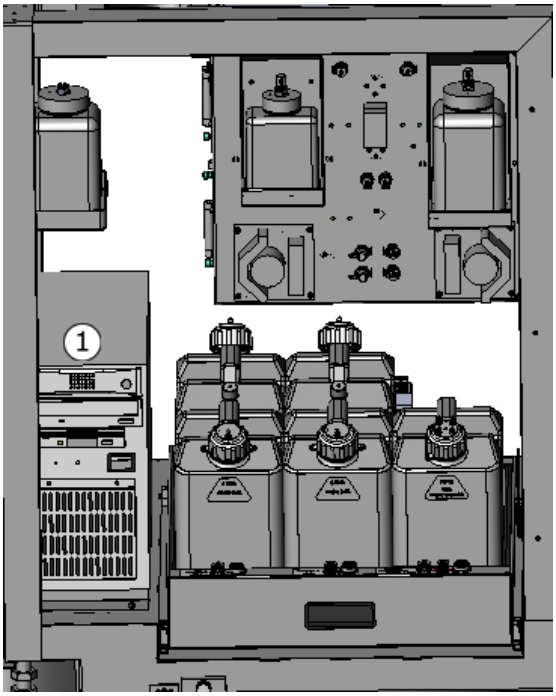


Figure 3-14: The System Computer

Position	Component
1	System Computer

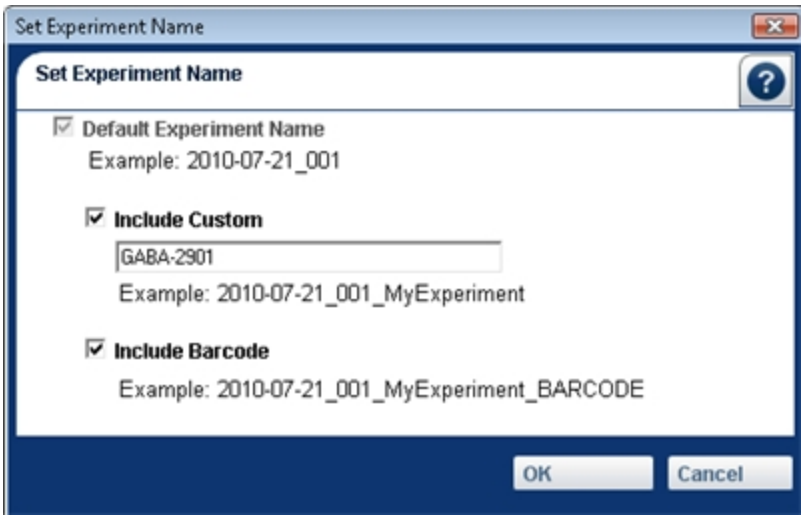
The Barcode Reader

The IonWorks Barracuda System includes a hand-held barcode reader for use with barcoded compound plates. Data file names can be appended with the barcode number as a method for organizing experiment results.

For detailed instructions on using the barcode reader, please see the manual included with the device.

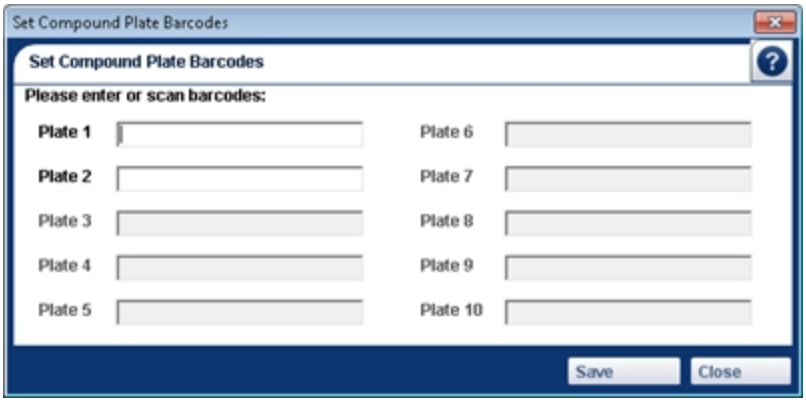
To use the barcode reader with the IonWorks Barracuda System:

1. Make sure the barcode reader is plugged into a USB port on the monitor.
2. Before running an experiment, click **Experiment > Experiment Name > Set**. The **Set Experiment Name** dialog appears.
3. Select the **Include Barcode** check box to append the barcode number to the folder name.



4. For your selected Channel Protocol, in the **Edit Channel Protocol > Aspirate > Barcode Position** field, make sure an option other than **NONE** is selected. See Specifying Aspirate Options on page 71.

   When you run an experiment, the **Set Compound Plate Barcodes** dialog appears immediately.



5. Scan the barcode labels as prompted.
6. Continue with normal data acquisition to complete the experiment.

Your data saves in a folder named whatever you specified in **Set Experiment Name** with your scanned barcode number at the end. See Setting the Experiment Name on page 118.

Chapter 4: Developing New Protocols

There are three types of protocols: Setup, Channel, and Cleanup. To run an assay, you must select one of each type of protocol. You can develop new protocols to match your lab standards by modifying the example protocols or by modifying any existing protocol. Select the protocol that most closely matches the assay type you need. Then modify the protocol using the protocol editor and save it as a new protocol. For Channel Protocols, you can run the protocol in Assay Development Mode, and then save it as a new protocol. Each example protocol provided by Molecular Devices also includes at least one sample metric for data analysis.

Note: Disabled by default, to enable the charge calculator features that are available in the **Edit Setup Protocol** dialog and **Edit Channel Protocol** dialog, select the **Enable Charge Calculator** check box in the **Preferences** dialog. See Estimating Experiment Total Charge on page 54 for details.

Developing Setup Protocols

The Setup Protocol begins the experiment with parameters for single-hole or PPC-type plate, hole test levels, amount and dispense speed for cell suspension, and seal test parameters after cells are added. The Setup Protocol also controls the steps for obtaining cell access and going whole cell. After the Setup Protocol completes, the Channel Protocol starts. Example Setup Protocols are available for single-hole (SH) and population patch clamp (PPC) plates.

For information about developing Setup Protocols, see Creating a Setup Protocol on page 55.

Developing Channel Protocols

Channel Protocols control data acquisition before, during, and after compound additions for ligand-gated or voltage-gated applications. The Channel Protocol parameters vary greatly depending on the type of assay you are running, the cell line, or the ion-channel of interest. Using the sample protocols, you can modify the parameters for developing an optimized assay. In Assay Development Mode, you can run through Channel Protocol scenarios and save the optimized assay protocol as a new protocol. After the Channel Protocol completes, the Cleanup Protocol starts. Example channel protocols include Voltage-Gated and Ligand-Gated protocols.

For information about developing Channel Protocols, see Creating a Channel Protocol on page 67.

Voltage-Gated Example Protocols

The following voltage-gated protocols involve one compound addition: channel blocker.

Type of Protocol	Listed in the Software
hERG - Potassium Channel	Example hERG Protocol Single Addition
Kv1.3 - Potassium Channel	Example Kv1.3 Protocol Single Addition
Nav1.5 - Sodium Channel	Example Nav1.5 Protocol Single Addition

Ligand-Gated Example Protocols

The following ligand-gated protocols involve two compound additions: channel blocker followed by channel activator:

Type of Protocol	Listed in the Software
GABA - GABA Receptor Ion Channel	Example Blocked GABA Protocol
ASIC1a - Acid Sensitive Ion Channel	Example Blocked ASIC1a Protocol
α7nACh - Nicotinic Acetylcholine Receptor Ion Channel	Example Blocked Alpha7 nAChR Protocol

Developing Cleanup Protocols

The Cleanup Protocol automates necessary maintenance steps between assays such as washing the pipettor tips, washing the CellPettor, and washing the electrodes on the Electrode-Plate. One standard example Cleanup Protocol is available.

For information about developing Cleanup Protocols, see Creating a Cleanup Protocol on page 97.

Experiment Mode

You can run experiments in the following modes:

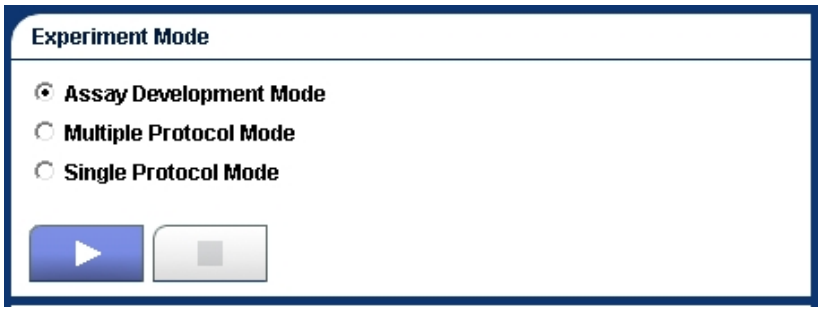
- **Assay Development Mode:** During Assay Development Mode, the selected Channel Protocol runs and pauses. At the pause, you can adjust parameters to modify the Channel Protocol. The assay continues to pause after each iteration of the Channel Protocol until you have optimized for your desired result. You can then save the optimized Channel Protocol as a new protocol, which can be run later in Multiple Protocol mode or Single Protocol mode. See Modifying a Channel Protocol in Assay Development Mode on page 47. If you are running in Assay Development Mode, follow the prompts at the pause to run through iterations of the Channel Protocol, or complete the experiment by proceeding to the Cleanup Protocol.
- **Multiple Protocol Mode**: In Multiple Protocol mode, you can select multiple Channel Protocols to run without interruption, except for specified pauses in operations to add or change compound plates or to add cells. Experiments are conducted using one Setup Protocol, any number of Channel Protocols, and one Cleanup Protocol.
- **Single Protocol Mode**: In Single Protocol Mode, the selected protocols run without interruption. One Setup Protocol, one Channel Protocol, and one Cleanup Protocol run through to completion, except for specified pauses in operations to add or change compound plates or to add cells.

Modifying a Channel Protocol in Assay Development Mode

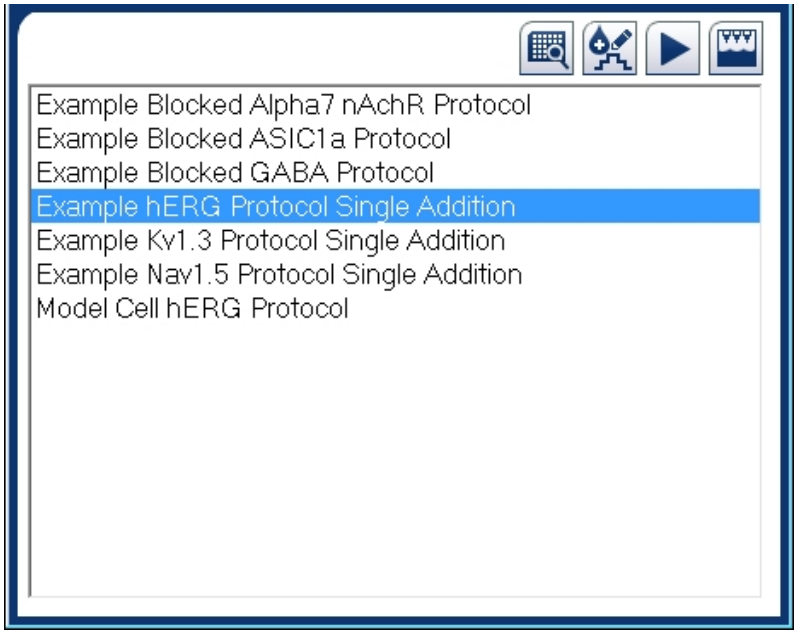
When you want to optimize a new type of assay, you can open a pre-defined Channel Protocol from Molecular Devices and then optimize the sample assay using Assay Development Mode.

To modify a pre-defined Channel Protocol in Assay Development Mode:

1. Select a Setup Protocol from the list of saved protocols.
2. Select a Cleanup Protocol from the list of saved protocols.
3. From the list of pre-defined Channel Protocols, select the Channel Protocol that most closely matches the assay you would like to optimize for.
4. To preview the parameters for the pre-defined protocol, or to make changes before running the protocol, click **Edit**. The Channel Protocol Editor appears.
5. Review the parameters. If applicable, make any necessary changes to the parameters listed for the Channel Protocol. Click **Save**. The Channel Protocol Editor closes.
6. In the Data Acquisition and Review workspace, click **Assay Development Mode**.



7. Click  to start the assay.
8. Add Experiment Notes, and then click **Continue**. See Adding and Viewing Experiment Notes on page 121. The instrument runs through the Setup Protocol and the Channel Protocol and then pauses. The Assay Development dialog appears.



9. Click  to review Channel Protocol data in the Data Acquisition and Review workspace. The plate view, Fixed Well view and Floating Well view functions are all active for your data review.
10. Click  to return to the Assay Development dialog.

11. Continuing depends on what you want to do next. You have the following choices:

Button	Task
	To edit a selected Channel Protocol: a. Click  to open the Channel Protocol Editor. b. Make any adjustments to the parameters, and then click **Save**. c. If the protocol you edited was marked Read-Only, then type a new Protocol Name and click **Save**.
	Run the selected Channel Protocol.

12. Click  when you are finished with the assay development. The selected Cleanup Protocol runs to end the experiment.

Using the Model Cell to Validate a Protocol

A companion component to the Electrode-Plate, the Model Cell simulates a whole cell circuit using resistors built into the frame housing. When you are developing new protocols, you can use the Model Cell in place of the Electrode-Plate to test the basic output and resistance of a voltage application before testing the protocol on actual cells.

The Model Cell is divided into two sides:

- The left bank of resistors (**A1**:**H24**) mimics a population patch clamp (PPC) PatchPlate at 200 kΩ. Fifty nano-amps (50 nA) of current is expected for every 10 mV applied in the protocol. Keep in mind that the currents for this bank easily reach the instrument limit (110 nA) for protocols run in single-hole (SH) mode.
- The right bank of resistors (**I1**:**P24**) mimics a single-hole (SH) PatchPlate at 10 MΩ. One nano-amp (1 nA) of current is expected for every 10 mV applied in the protocol.

When you review the data from a Model Cell assay, keep in mind that the two types of resistors produce different results, some of which might not apply to your intended test.

To use the Model Cell to validate a voltage protocol:

1. Select Setup, Channel, and Cleanup Protocols.
2. Place the Model Cell unit on the wash station in place of the Electrode-Plate.
3. Start the experiment in Assay Development Mode if you want to adjust the voltage parameters.
4. To switch between SH and PPC testing, change the PatchPlate Type selection in the Setup Protocol, then run the assay again. Expect current saturation in all wells served by the PPC resistor bank when running a SH protocol and applying voltage less than –25 mV or greater than +25 mV.

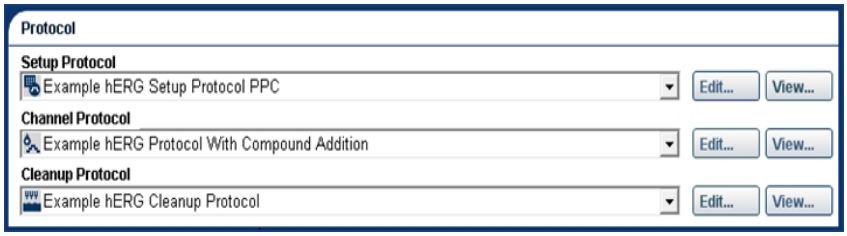
Saving Read-Only Protocols

The sample protocols are marked as read-only, and they cannot be modified or saved. If you change the protocol name, however, you can save the protocol settings as a new protocol. You can then modify the protocol parameters to suit your needs.

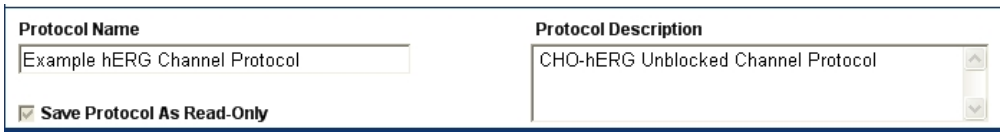
In addition, you can mark any protocol as read-only to prevent accidental changes.

To save a read-only protocol with a new name:

1. In the Protocols section of the Data Acquisition and Review Workspace, select a read-only protocol selection field. See Data Acquisition and Review Workspace on page 15.



2. Click **Edit** to open the Protocol Editor.
3. If the **Save Protocol as Read-Only** check box is active, clear the check box.



4. If the protocol is an example protocol and the **Save Protocol as Read-Only** check box is not active, type a new name in the **Protocol Name** field. The protocol name can include up to 100 characters. Do not include the following characters since they are invalid: **\ / : * ? " < > |**.
5. In the protocol description field, you can type any notes or information about the protocol that you want to record. This field allows up to 250 text characters.
6. Select the **Save Protocol as Read-Only** check box to prevent future changes to the protocol. Leave the box unchecked to allow modifications to the protocol in the future.
7. Click **Save**. The new protocol appears in the **Protocols** field.

Viewing the Protocol Summary

On the **Review Protocol** tab, you can view a summary of the all the parameters defined in the steps for the protocol.

To view the protocol summary:

1. In the Protocol section of the Data Acquisition and Review workspace, select a protocol from the protocol selection field. For details, see Data Acquisition and Review Workspace on page 15.
2. Click **Edit** to open the Protocol Editor.
3. Click  in the protocol editor.
4. To change any of the defined parameters, return to the step-tab for the parameter and make your changes.
5. To print the summary click  and follow the prompts.
6. To copy the summary to the clipboard, click  . The summary data is copied to the clipboard. Paste the data into your preferred application.

Increasing Assay Window Longevity

Molecular Devices scientists have found that refreshing the recording buffers just prior to recording ionic currents (“buffer wash”) can greatly increase the useable assay window for stable ionic current magnitudes on the IonWorks Barracuda System. For best results, we recommend exchanging the internal and external buffers just prior to adding cells and then exchanging the external solution just after cell seal formation prior to recording ionic currents. This can be done automatically by configuring these exchanges in the **Setup Protocol**.

Recommended buffer wash procedures include exchanging buffers before the cell addition and after the cell addition. Set buffer washes while creating a Setup Protocol in the **Edit Setup Protocol** dialog. See Setting Buffer Wash Recommendations on page 51 for procedures.

Setting Buffer Wash Recommendations

Molecular Devices recommends the following buffer wash setting guidelines for both **Buffer Wash Before Cell Addition** and **Buffer Wash After Cell Addition**. Set buffer washes while creating a Setup Protocol in the **Edit Setup Protocol** dialog.

Note: Different cell lines or ion channel targets may require different settings than these recommended guidelines.

Recommended Settings for Buffer Wash Before Cell Addition

To configure the buffer wash before cell addition settings:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the **Edit Setup Protocol** dialog.
3. In the **Add Cells** section of the **Step 1** tab, select the **Wash before cell addition** check box to run one or more external buffer and internal buffer exchanges before cells are added. Click ... to open the **Buffer Wash Before Cell Addition** dialog to configure the wash.
4. In the **Buffer Wash Before Cell Addition** dialog, enter the settings provided in the dialog figure below.

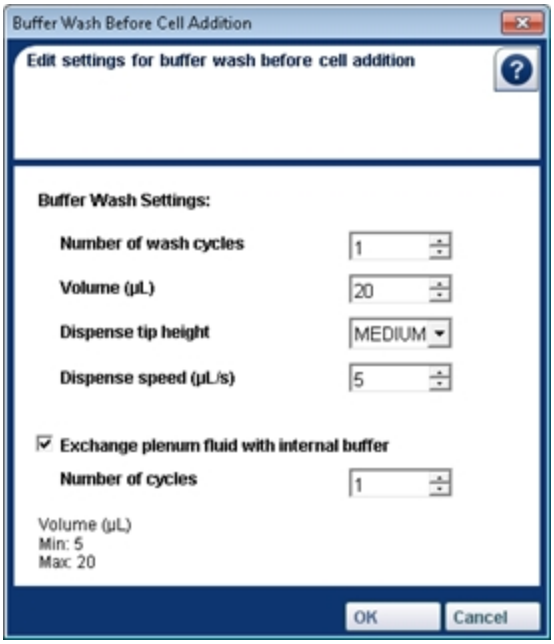


Figure 4-1: Recommended Buffer Wash Before Cell Addition Settings

5. Click **OK** when done.

Recommended Settings for Buffer Wash After Cell Addition

To configure the buffer wash after cell addition settings:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the **Edit Setup Protocol** dialog.
3. In the **Seal test** section of the **Step 1** tab, select the **Wash after cell addition** check box to run one or more external buffer and amphotericin exchanges after cells are added. Click  to open the **Buffer Wash After Cell Addition** dialog to configure the wash.
4. In the **Buffer Wash After Cell Addition** dialog, enter the settings provided in the dialog figure below.

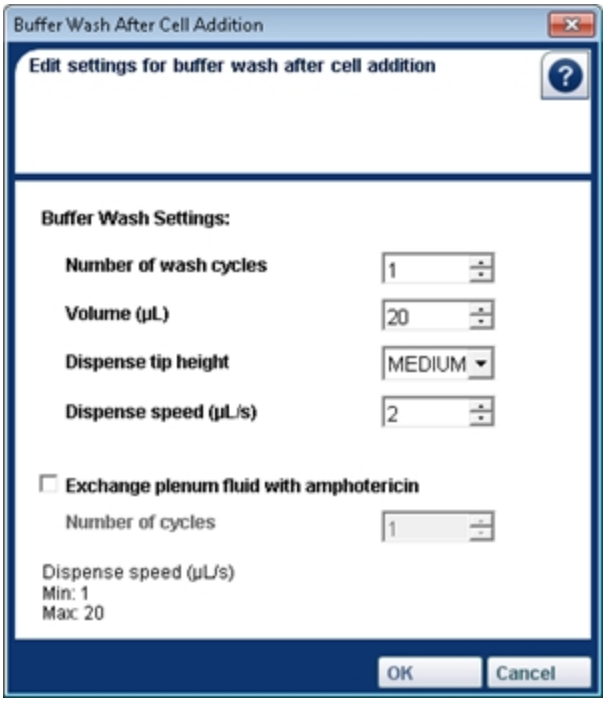


Figure 4-2: Recommended Buffer Wash After Cell Addition Settings

5. Click **OK** when done.

Estimating Experiment Total Charge

The Coulomb Meter in the software calculates the total charge passed during a typical experiment. The calculation value can be used to troubleshoot technical or assay issues. The Coulomb Meter is located in the upper right corner of the **Edit Setup Protocol** dialog and the **Edit Channel Protocol** dialog. The total charge is estimated by taking the integral of the calculated current using the voltage protocol and the seal resistance value specified in the **Estimated Seal Resistance** field, identified by the orange border.

Figure 4-3: Charge Calculator Features in the Edit Setup Protocol Dialog

The input value for **Estimated Seal Resistance** is for calculating the anticipated total charge, and does not represent the actual experiment seal resistance.

Note: To enable the charge calculator features, select the **Enable Charge Calculator** check box in the **Preferences** dialog.

Chapter 5: Creating a Setup Protocol

5

The Setup Protocol begins the experiment with parameters for single-hole or PPC-type plate, hole test levels, amount and dispense speed for cell suspension, and seal test parameters after cells are added. The Setup Protocol also controls the steps for obtaining cell access and going whole cell. After the Setup Protocol completes, the Channel Protocol starts.

To create a Setup Protocol:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.

2. Click **Edit** to open the **Edit Setup Protocol** dialog.
3. Review the parameters in each of the protocol editor steps:
   - Step 1: Specifying Plates, Tests, and Cell Access on page 56
   - Step 2: Editing Native Filters on page 65
   - Viewing the Protocol Summary on page 51
4. When you have finished setting the parameters, click **Save** to save your changes and to close the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.

Step 1: Specifying Plates, Tests, and Cell Access

The first step in creating a setup protocol is to specify the **Plates Type, Test Levels, and Cell Access Steps** to use in the protocol.

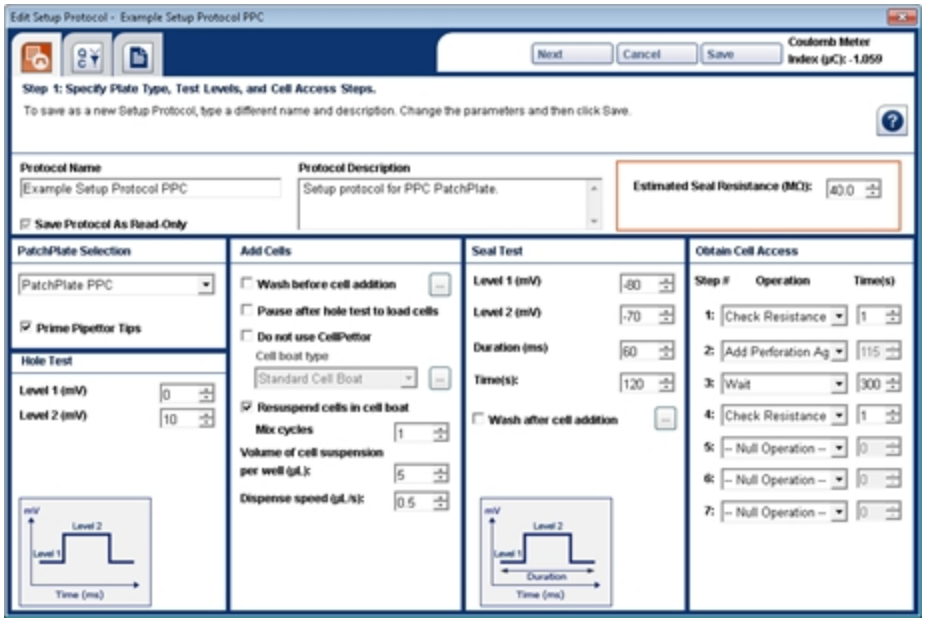


Figure 5-1: Edit Setup Protocol Step 1 Tab Dialog

To specify plate type, test levels, and cell access steps:

1. In the **Protocol** section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.

2. On the Step 1 tab, specify the parameters to use. For details, see:

- Selecting the PatchPlate Type on page 57
- Setting Hole Test Voltage Levels on page 58
- Adding Cells on page 59
- Setting Seal Test Voltage Levels on page 62
- Obtaining Cell Access on page 64

When typing values in the **Hole Test**, **Add Cells**, **Seal Test**, and **Obtain Cell Access** sections, the valid value range for the active field appears in the lower-left corner of the section. When you type an invalid value in a field, an asterisk appears next to the field and the value is shown in red type. An error message appears in the information area near the top of the window.

If you enter an invalid value, the protocol cannot be saved until the invalid entry is corrected.

Note: To enable the charge calculator features, select the **Enable Charge Calculator** check box in the **Preferences** dialog.

3. When you have finished configuring the parameters, click **Next** to go to the next tab, or click **Save** to close the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.

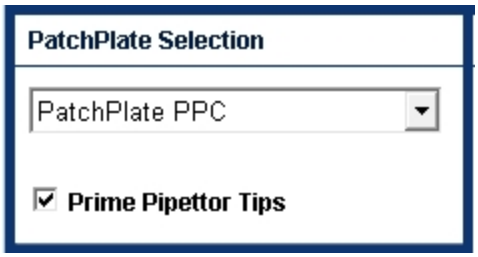
Selecting the PatchPlate Type

The PatchPlate is a 384-well consumable microplate from Molecular Devices for partitioning and positioning the cells. The PatchPlate type is set in the Setup Protocol.

To select the PatchPlate type:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. In the PatchPlate section of the Step 1 tab, select the type of consumable PatchPlate you are using for this experiment, and specify if the Pipettor tips are being reused and need priming. For details, see Priming Pipettor Tips on page 58.

- PatchPlate SH (384-wells, single-hole wells, use to obtain MegaSeal planar patch-clamp recordings)
- PatchPlate PPC (384-wells, 64-holes per well, population patch clamp, use to obtain MegaSeal planar patch-clamp recordings)



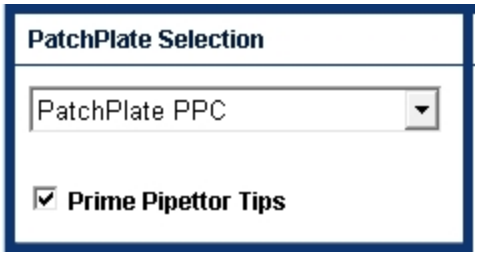
4. Click **Save**. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.

Priming Pipettor Tips

The Pipettor aspirates and dispenses fluids in precise amounts according to instructions specified in the software protocols. The disposable Pipettor tips can be washed between runs for reuse as specified in the Cleanup Protocol. However, when the Pipettor tips are reused, air bubbles can be dispensed. To reduce the risk of dispensing air bubbles, Molecular Devices recommends specifying **Prime Pipettor Tips** in your Setup Protocol. The Pipettor tip priming operations involves a special cycle of aspirating and dispensing external buffer several times before adding buffer to the PatchPlate during an experiment run.

To specifying the Pipettor tip priming:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. In the PatchPlate section of the Step 1 tab, check the **Prime Pipettor Tips** option.



Setting Hole Test Voltage Levels

During the Hole Test, the Electrode-Plate records the electrical current after the wells are filled with external buffer, but before cells have been added. A well is defined as **Blocked** when the measured resistance is less than 1 MΩ or greater than 10 MΩ. Wells that do not pass the hole test are excluded when data is analyzed.

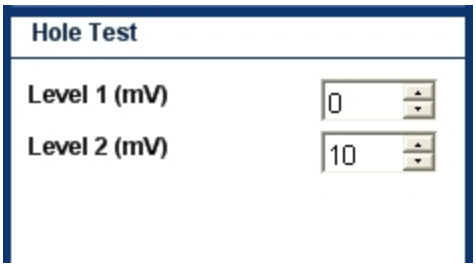
The Hole Test parameters are set in the Setup Protocol.

To set parameters for the Hole Test:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.

2. Click **Edit** to open the Protocol Editor.
3. In the Hole Test section of the Step 1 tab, set these parameters:

- **Level 1** field, type the value in millivolts for the initial level.
- **Level 2** field, type the value in millivolts to step to after the Level 1 voltage is applied.



4. Click **Save**. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.

Adding Cells

You define the way cells are added to the experiment in the **Obtain Cell Access** section of the **Edit Setup Protocol** dialog.

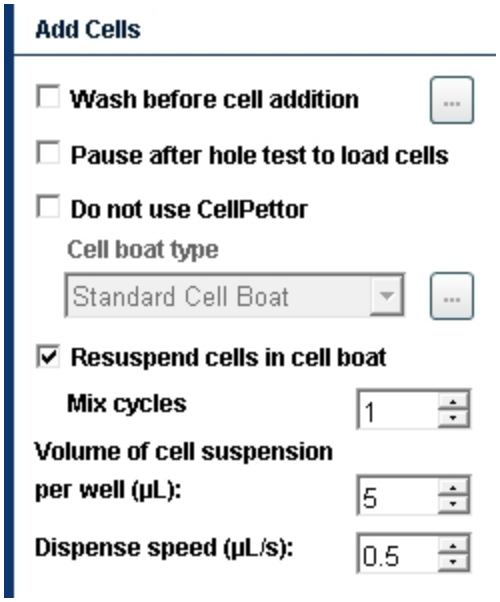
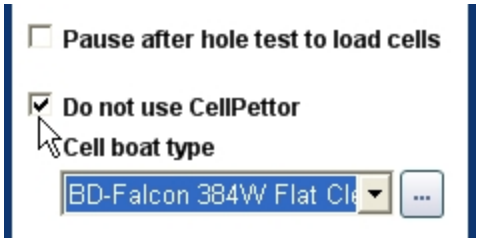


Figure 5-2: Add Cells section of the Step 1 Tab

To define cell additions:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.

3. In the **Add Cells** section of the **Step 1** tab, select the **Wash before cell addition** check box to run one or more external buffer and internal buffer exchanges before cells are added. Click ... to open the **Buffer Wash Before Cell Addition** dialog to configure the wash. See Setting Buffer Wash Before Cell Addition on page 60 for details.
4. Select the **Pause after hole test to load cells** check box to require the system to pause the experiment so that you can position the prepared cell plate on the process deck. When you are finished, click **Continue** to proceed with the experiment in progress.
5. To manually add cells to the cell boat and disable the cell-handling components, select the **Do not use CellPettor** check box.



- Select an allowed cell boat type so that the instrument can correctly position the pipettors in the cell boat. Contact Molecular Devices Technical Support to request additional boat types.
- To view details about the selected cell boat type, such as Manufacturer's Part Number or precise cell boat dimensions, click ... to open the **View Plate Definition** dialog which includes details about the boat such as part numbers and precise dimensions. See Viewing Plate Definitions on page 61 for details.

  The CellPettor cell-handling subsystem requires the use of Molecular Devices approved cell boats [Standard cell boat: Molecular Devices PN 5005252]. Using labware other than the Molecular Devices approved cell boats could damage the CellPettor.

6. Select the **Resuspend cells in cell boat** check box to enable the **Mix cycles** field. To resuspend the cells in the cell boat, the pipettors aspirate and dispense small amounts of cell preparation. Each aspirate and dispense combination is one mix cycle.

   If you have selected this option, then select the number of **Mix cycles** the instrument should perform.
7. In the **Volume of Cell Suspension per well** field, type the number of microliters the pipettors should aspirate and transfer to the PatchPlate.
8. In the **Dispense Speed** field, type the number of microliters per second of cell suspension the pipettors should dispense to the PatchPlate.
9. Click **Save**. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.

Setting Buffer Wash Before Cell Addition

In the **Add Cells** section of the **Step 1** tab, select the **Wash before cell addition** check box to run one or more external buffer and internal buffer exchanges before cells are added.

Configure the plenum wash settings in the **Buffer Wash Before Cell Addition** dialog.

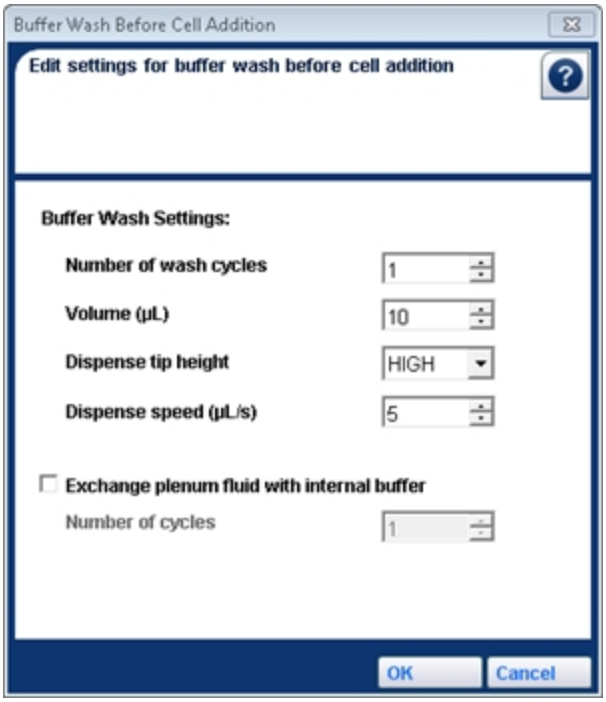


Figure 5-3: Buffer Wash Before Cell Addition settings dialog

The **Buffer Wash Settings** section allows you to:

- Specify the **Number of wash cycles** using external buffer, **Volume dispensed**, **Dispense tip height**, and **Dispense speed**.
- Choose to **Exchange plenum fluid with internal buffer**, and specify the **Number of cycles** the fluid is exchanged.

Viewing Plate Definitions

The information listed for plate definitions is provided for reference only and cannot be edited. Volumes can be used to calculate aspiration and dispense parameters. The Manufacturer's part number is listed for ordering purposes.

Figure 5-4: Sample View Plate Definition Dialog

CAUTION! Use only approved cell and compound boats or damage to the pipettors could occur.

CAUTION! Fill the boat or plate with adequate volume. Inadequate volume can cause the pipettor head to aspirate an air and fluid mixture that can ruin an experiment by dispensing air into the PatchPlate wells.

Setting Seal Test Voltage Levels

The seal test determines whether the cell is properly positioned over the hole and sealed in the well. If the cell is not properly sealed as designated by the native filter settings, then the values for that well are excluded from analysis.

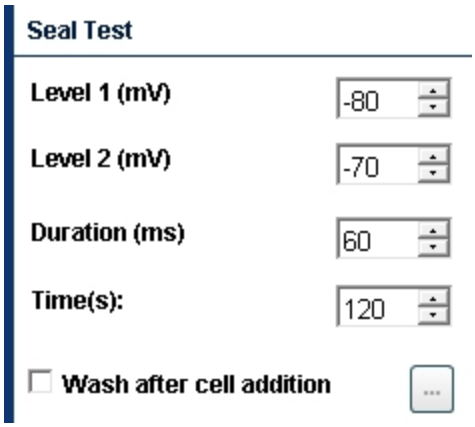


Figure 5-5: Seal Test section of the Step 1 Tab

To set seal test parameters:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the **Edit Setup Protocol** dialog.
3. In the **Step 1** tab in the **Seal Test** section, set these parameters:

- **Level 1**: The holding level voltage for the seal test.
- **Level 2**: The pulse voltage for the seal test.
- **Duration**: The length in milliseconds of voltage application for the seal test. The duration value can be in the range of 40 ms to 600 ms.
- **Time**: The length of time in seconds to continue applying the seal test voltage repetitions.
- **Wash after cell addition**: Run one or more external buffer and amphotericin exchanges after cells are added. Click ... to open the **Buffer Wash After Cell Addition** dialog to configure the wash. See Setting Buffer Wash After Cell Addition on page 63 for details.

4. Click **Save**. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.

Setting Buffer Wash After Cell Addition

In the **Seal test** section of the **Step 1** tab, select the **Wash after cell addition** check box to run one or more external buffer and amphotericin exchanges after cells are added.

Configure the plenum wash settings in the **Buffer Wash After Cell Addition** dialog.

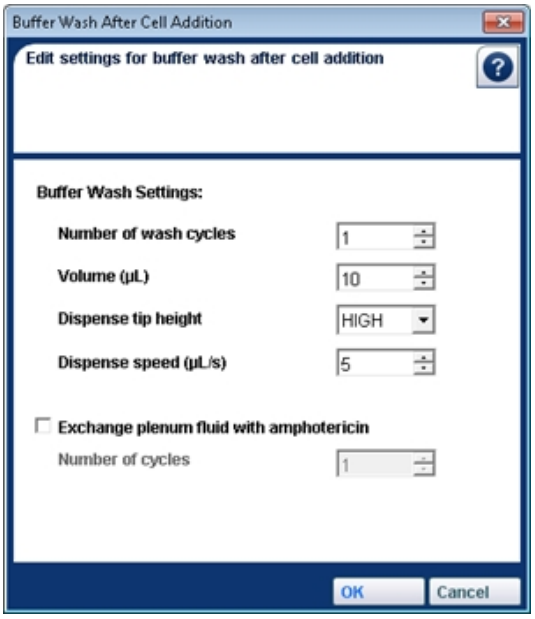


Figure 5-6: Buffer Wash After Cell Addition settings dialog

The **Buffer Wash Settings** section allows you to:

- Specify the **Number of wash cycles** using external buffer, **Volume dispensed**, **Dispense tip height**, and **Dispense speed**.
- Choose to **Exchange plenum fluid with amphotericin**, and specify the **Number of cycles** the fluid is exchanged.

Obtaining Cell Access

You can set up to seven steps for the sequence of operations to obtain cell access.

To set cell access parameters:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the **Edit Setup Protocol** dialog.
3. In the **Step 1** tab in the **Obtain Cell Access** section, set up to seven steps:

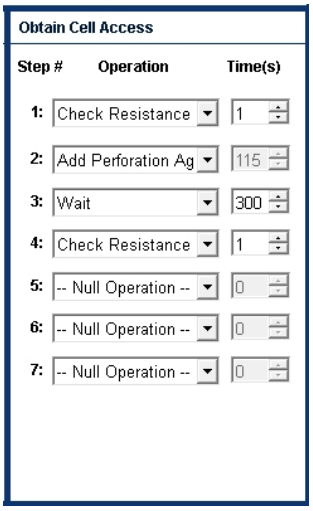


Figure 5-7: Seven Operation and Time(s) fields in the Obtain Cell Access Section

Each step can be configured as one of the following step types:

- **Check Resistance:** Rerun the Seal Test and specify the duration in the range of 1 to 60 seconds.
- **Add Perforation Agent:** Introduce the cell perforation agent into the plenum. This operation has a fixed duration of 115 seconds.
- **Add Internal Buffer:** Prime the plenum with internal buffer. This operation has a fixed duration of 70 seconds.
- **Wait:** Pause the experiment for 60 to 600 seconds.
- **Null Operation:** Take no action. Used for undesignated steps in the series.

4. Click **Save**. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.

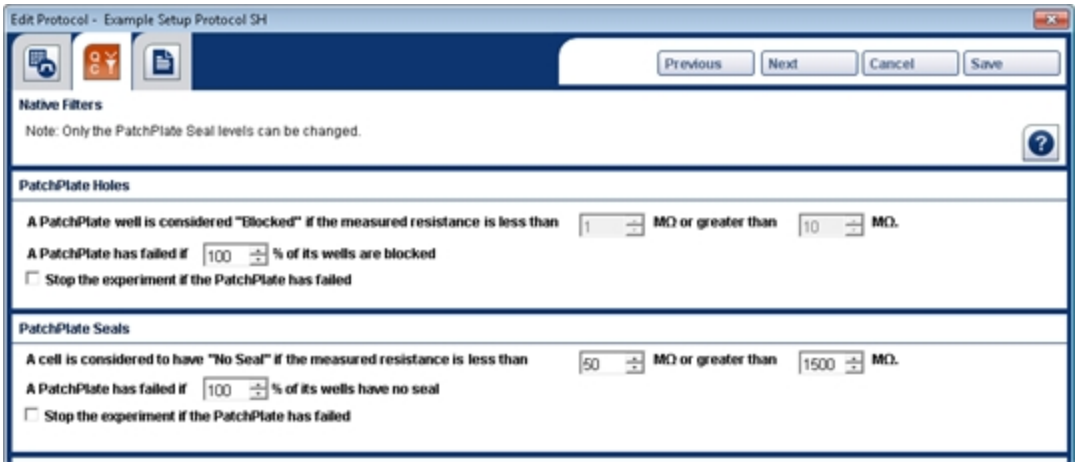
Step 2: Editing Native Filters

You can view filtering parameters for the hole test and set filtering parameters for the seal test. Wells that do not pass native filter tests are excluded from data analysis.

To view or edit hole test and seal test parameters:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Setup Protocol from the **Setup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. Click the **Step 2: Editing Native Filters** tab, and then specify the following parameters:

- **PatchPlate Holes:** The hole test filter measures for proper resistance levels between the electrodes and the plenum for each well. Resistance that measures less than 1 MΩ or greater than 10 MΩ indicates the well is blocked. Resistance that is too high might mean there are air bubbles in the well. The hole test thresholds are pre-defined and cannot be changed for PatchPlate quality control purposes. You can specify when a PatchPlate fails by setting the percentage of wells blocked threshold. You can automatically stop the experiment by selecting the **Stop the experiment if the PatchPlate has failed** check box. When the Stop check box is clear, the experiment continues to run and a QC fail message appears in the status bar.
- **PatchPlate Seals:** The seal test filter measures that the cells are properly sealed on the holes in the PatchPlate wells. When data files are acquired, the default definition for 'No Seal' is defined as resistance that is less than 50 MΩ for SH pates or 20 MΩ for PPC plates, or greater than 1500 MΩ for either SH plates or PPC plates. You can change these settings for a given data file (ranging from 1 to 100,000 MΩ) to increase or decrease the stringency of the native filters. You can specify when a PatchPlate fails by setting the percentage of wells with no seals threshold. You can automatically stop the experiment by selecting the **Stop the experiment if the PatchPlate has failed** check box. When the Stop check box is clear, the experiment continues to run and a QC fail message appears in the status bar.



4. When you have finished configuring the parameters, click **Next** to go to the next tab, or click **Save** to close the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.

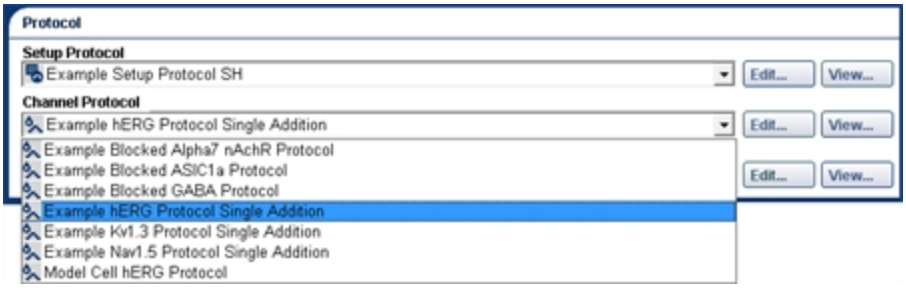
Chapter 6: Creating a Channel Protocol

6

Channel Protocols control data acquisition before, during, and after compound additions for ligand-gated or voltage-gated applications. The Channel Protocol parameters vary greatly depending on the type of assay you are running, the cell line, or the ion-channel of interest. Using the sample protocols, you can modify the parameters for developing an optimized assay. In Assay Development Mode, you can run through Channel Protocol scenarios and save the optimized assay protocol as a new protocol. After the Channel Protocol completes, the Cleanup Protocol starts.

To create a Channel Protocol:

1. In the Protocol section of the Data Acquisition and Review workspace, in the **Experiment Mode** section, if not already selected, select **Single Protocol Mode**.
2. In the **Protocol** section, select a **Channel Protocol** . See Data Acquisition and Review Workspace on page 15 for details.



3. Click **Edit** to open the **Edit Channel Protocol** dialog.
4. Review the parameters in each of the protocol editor steps, and change parameters as applicable for the current experiment.
5. Click **Save** to finish. If **Save Protocol As Read-Only** has been selected, change the **Protocol Name** and then click **Save**.

Note: The single Channel Protocols are available for inclusion in a Multiple Channel Protocol. See Creating Multiple Channel Protocols on page 93.

Step 1: Specifying Scans, Fluid Transfers and Compound Additions

You define parameters for baseline scans in the **Step 1: Specifying Scans and Compound Additions** tab of the Channel Protocol editor.

Specifying Scan Parameters

To set the parameters for scans:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. On the first tab window in the **Edit Channel Protocol** dialog, specify the following parameters:

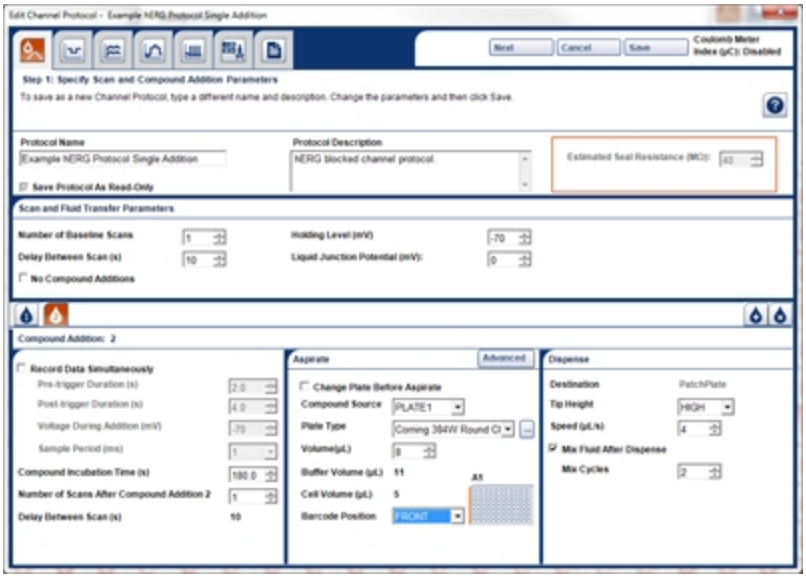


Figure 6-1: Edit Channel Protocol dialog

- **Number of Baseline Scans**: The number of baseline scans (range 0 to 10) required for the experiment. The baseline scan measures ion channel current before the addition of any compound.
- **Delay Between Scans**: The number of seconds between scans, including baseline and all pre-compound and post-compound addition scans. Type the number of seconds between scans required for the experiment.
- **Holding Level**: The voltage applied before the pre-signal and after the command voltage. Type the desired holding level voltage for the experiment.

- **Liquid Junction Potential**: The value you enter to correct for liquid junction potential shifts. Calculate the value and type your result in millivolts in the Liquid Junction Potential field. The value input in the Liquid Junction Potential Correction field will be subtracted from the command voltage.

  Note: Liquid-junction potentials (defined as the potential differences between two solutions) occur at all boundaries between solutions of different compositions. The IonWorks Barracuda System zeros all the voltage offsets that arise from electrode to solution junction potentials and liquid-junction potentials during the first portion of the experiment prior to the cell addition. However, the Liquid Junction Potential is then at least partially removed by cell addition and occlusion of the holes resulting in the command voltages being off by approximately the value of the Liquid Junction Potential.

  The final correction factor that is applied to compensate for these voltage-offsets during cell recordings requires a user-defined value for the Liquid Junction Potential Correction. The voltage-offset values (if significant) must be determined empirically for each combination of external and internal solutions. This can be determined by performing an experiment run with a particular solution pair with 0 mV input as the Liquid Junction Potential Correction. After the experiment run, the value of the baseline current can be measured using user-defined metrics and any voltage error can be determined using Ohms Law, the baseline current value and the command voltage value. Generally these corrections are minor and 0 mV can be used.

- **No Compound Additions**: Run the baseline scan and complete the Channel Protocol with no compound additions. When this check box is selected, the experiment concludes after the last baseline scan completes. The compound additions, including the option to record data simultaneously, are not available.
- **Record Data Simultaneously**: Enable compound-triggered data acquisition for ligand-gated assays. This option is available only if you clear the No Compound Additions check box.

4. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Set related parameters. For instructions, see:

  Specifying Compound Additions on page 70

  Specifying Aspirate Options on page 71

  Specifying Advanced Aspirate Options on page 73

  Specifying Dispense Options on page 74
- Click **Next** to edit other Channel Protocol settings.

Specifying Compound Additions

In the **Compound Addition** section you specify the parameters for compound additions in the experiment. When more than one compound is added, the fluid levels in the well are maintained with subsequent additions.

To set parameters for compound additions:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. On the Step 1 tab in the protocol editor, in the compound additions section, specify the following parameters:

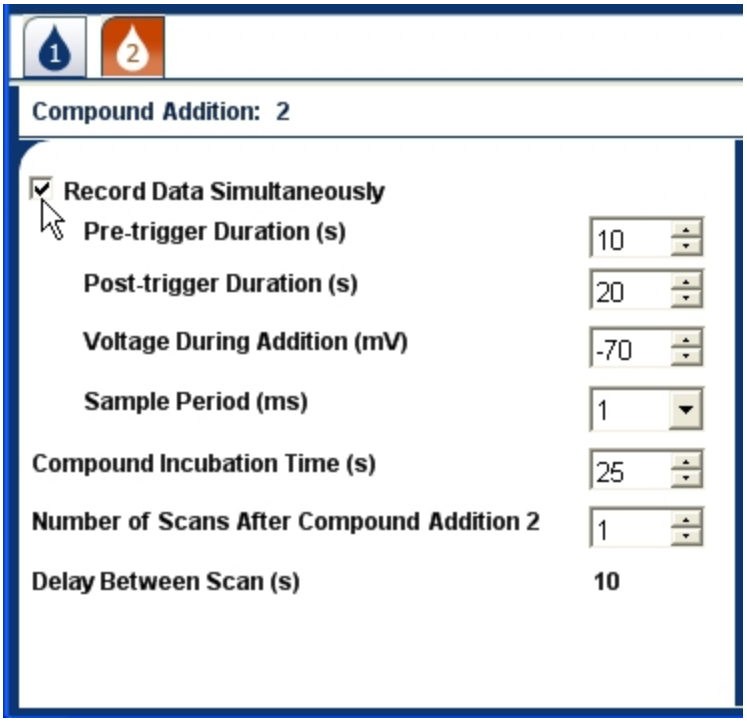


Figure 6-2: Compound Addition Section of the Edit Channel Protocol Dialog

- Select the **Record Data Simultaneously** check box to record data as the compound is being added.
- **Pre-Trigger Duration**: Type the time in seconds that data will be collected prior to compound addition.
- **Post-Trigger Duration**: Type the time in seconds that data will be collected after compound addition.
- **Voltage During Addition**: Type the voltage to be applied to the cell during compound-triggered acquisition.

- **Sample Period**: Select the length of time for the data sampling period from the Sampling Period field in milliseconds per sample.
- **Compound Incubation Time**: Type the total number of seconds the system should wait before the next scan. This value is the total time between scans and includes any time specified for the Post-Trigger Duration. The value entered for the Compound Incubation Time must be greater than the Post-Trigger Duration value, plus an additional 5 seconds for software processing time.
- **Number of Scans After Compound Addition**: Specify the number of times data is collected after each compound addition.
- **Delay Between Scans**: The number of seconds between scans after each compound addition.

Note: The Delay Between Scans is displayed here for reference only. The delay value for all scans is set in the Scan and Fluid Transfer Parameters area.

4. (Optional) Click  at far right of the section bar to add an additional compound, and then specify parameters.

Note: Maximum number of compound additions is 10.

5. (Optional) Select an additional compound to delete, and then click  at far right of the section bar.
6. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Specify related parameters. For instructions, see:
  Specifying Aspirate Options on page 71
  Specifying Advanced Aspirate Options on page 73
  Specifying Dispense Options on page 74
- Click **Next** to edit other Channel Protocol settings.

Specifying Aspirate Options

In the **Aspirate** section of the Channel Protocol, specify aspiration methods for the compound.

To set parameters for aspirating compound:

1. In the **Protocol** section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.

2. Click **Edit** to open the **Edit Channel Protocol** dialog.
3. On the first tab window in the **Aspirate** section, specify the following parameters:

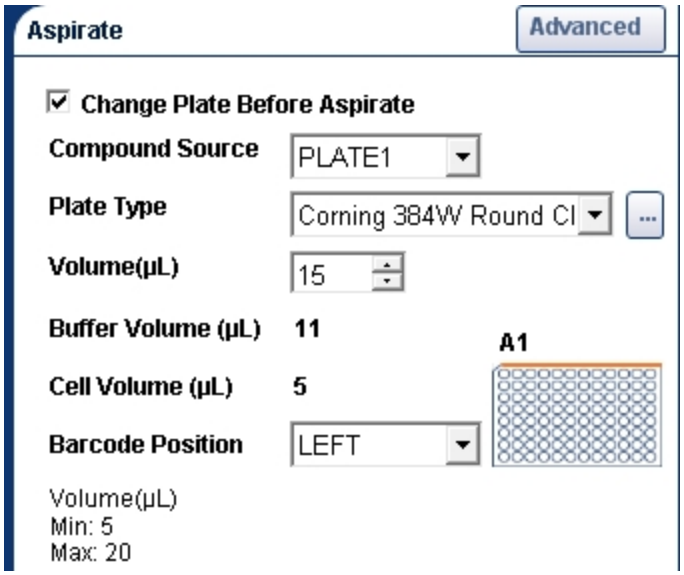


Figure 6-3: Aspirate Section of the Edit Channel Protocol Dialog

- Select the **Change Plate Before Aspirate** check box if you want the system to pause the experiment so that you can change the compound plate. When the system pauses, it is safe to open the instrument cover and change plates. After changing the plate, click **Continue** to resume the experiment.
- **Compound Source**: Select the location on the process deck for the chosen compound: Compound 1 or Compound 2. For three or more compound additions, replace the compounds in the Compound 1 and Compound 2 positions on the deck and set parameters as applicable.
- **Plate Type**: Click the list and select the manufacturer and type of plate used for delivery of the compound. If the plate you are using does not appear in the list, call Molecular Devices technical support for help adding the plate to the list.

  For detailed dimensions to use in calculating compound volumes, click ... to open the **View Plate Definition** dialog.
- **Volume**: Type the amount of fluid in microliters to be aspirated from the compound plate well.
- **Barcode Position**: Select the location of the barcode label on the compound plate: None, Front, Back, Left or Right.

  Note: If you selected **Experiment Name > Set > Include Barcode**, **NONE** cannot be the selected barcode position. See Setting the Experiment Name on page 118.
- Click **Advanced** to specify more detail for aspiration in the **Advanced Aspirate** dialog. See Specifying Advanced Aspirate Options on page 73.

4. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Specify other related parameters. For instructions, see:
  Specifying Advanced Aspirate Options on page 73
  Specifying Dispense Options on page 74
- Click **Next** to edit other Channel Protocol settings.

Specifying Advanced Aspirate Options

Set advanced parameters of the compound aspiration by clicking the **Advanced** button in the **Aspirate** section of the Channel Protocol editor.

To set advanced parameters for aspirating compound:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. On the Step 1 tab in the Aspirate section, specify the following parameters:

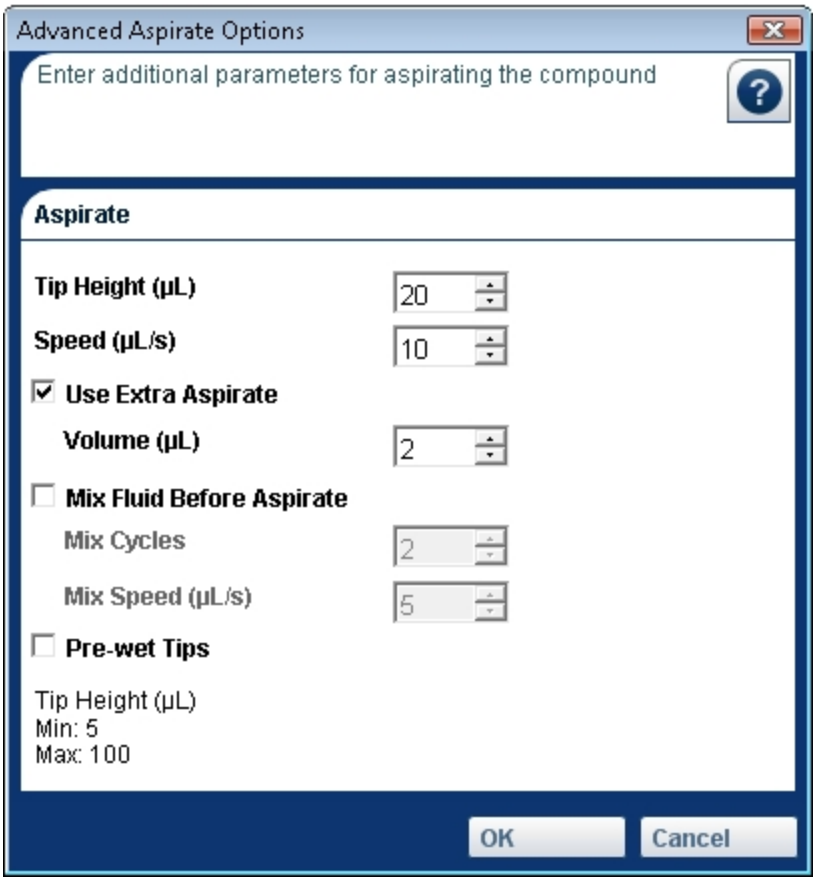


Figure 6-4: Advanced Aspirate Options Dialog

- Adjust the **Tip Height** to specify the volume in microliters to specify the level to which the pipettor descends from the well bottom. The pre-defined value is 10 µL.
- Adjust the **Speed** to specify the number of microliters per second the pipettor aspirates. The pre-defined value is 15 µL/s.
- Select the **Use Extra Aspirate** check box to draw extra fluid from the compound plate well. When this check box is selected, the **Volume** entry field is enabled. Type the number of extra microliters per well to be aspirated in the **Volume** field.
- Use the Extra Aspirate option to avoid dispensing air into the wells of the PatchPlate. When Extra Aspirate is enabled, the pipettors dispense the initial compound volume to the PatchPlate, then dispense the extra compound volume to the wash station.
- Select the **Mix Fluid before Aspirate** check box to configure the system to aspirate and dispense compound in the compound plate before aspiration for delivery to the PatchPlate. When this check box is selected, the **Mix Cycles** and **Mix Speed** fields are enabled. Type the number of mix cycles and the number of microliters of fluid to be aspirated and re-dispensed.
- Select the **Pre-wet Tips** check box to instruct the system to pre-wet the tips of the pipettors with compound before performing the aspirate operation. Make sure you have adequate compound volume to cover this step.
- Use the **Pre-wet Tips** option to reduce non-specific binding of sticky compounds in the pipettor tips. When the Pre-wet Tips option is enabled, the pipettors aspirate an initial volume of compound and dispense it to the wash station before aspirating the actual volume and dispensing to the PatchPlate wells so that the binding occurs on the first disposable aspirate cycle.

4. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Specify related parameters. For instructions, see:
  Specifying Aspirate Options on page 71
  Specifying Dispense Options on page 74
- Click **Next** to edit other Channel Protocol settings.

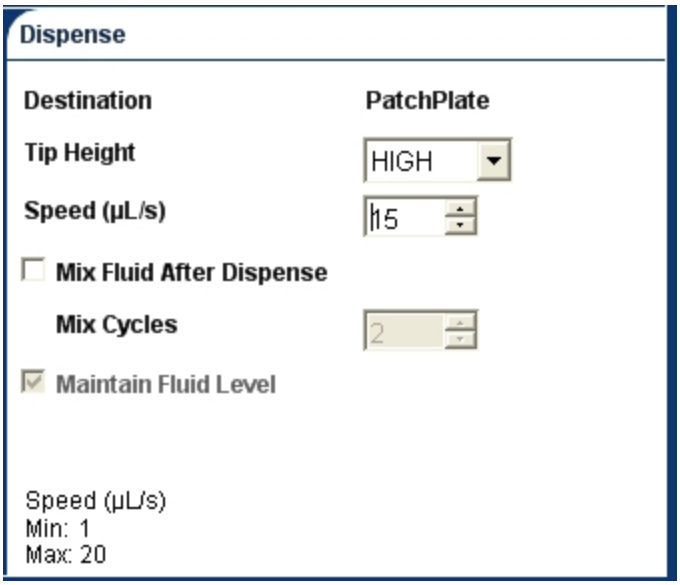
Specifying Dispense Options

Specify the details of the compound dispense operation in the **Dispense** section of the Channel Protocol editor. For each compound addition, the instrument maintains the fluid levels in the wells for each new compound addition.

To specify dispense options:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.

2. Click **Edit** to open the Protocol Editor.
3. On the Step 1 tab in the Dispense section, specify the following parameters:



- **Tip Height**: Select Low, Med, or High for the height of the tip insertion into the well.
- **Speed**: Type the number of microliters per second that should be dispensed from the pipettor.
- **Mix Fluids After Dispense**: Select this check box to enable mixing.
- **Mix Cycles** and **Volume**: Type the number of mix cycles and the volume of fluid to be mixed.

4. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Specify related parameters. For instructions, see:
  Specifying Aspirate Options on page 71
  Specifying Advanced Aspirate Options on page 73
- Click **Next** to edit other Channel Protocol settings.

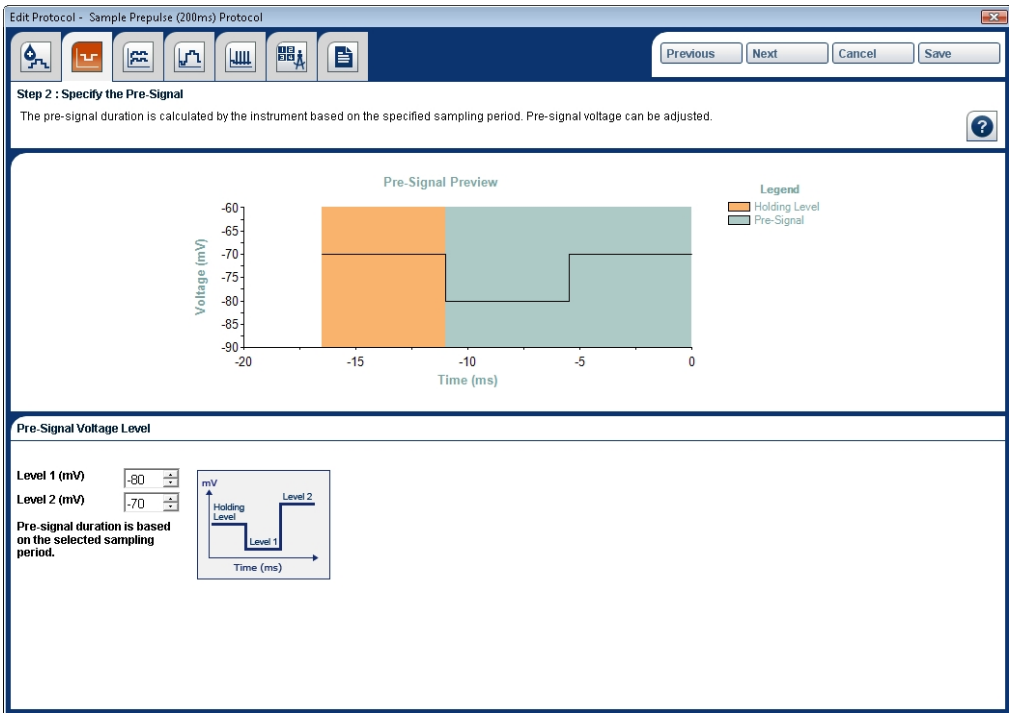
Step 2: Specifying the Pre-Signal

You can adjust the pre-signal voltage for calculating leak correction before data is acquired. The duration of the pre-signal is automatically calculated based on the selected sampling rate and always consists of a total of 150 sample points.

To specify the pre-signal voltage:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.

2. Click **Edit** to open the Protocol Editor.
3. On the Step 2 tab in the Pre-Signal Voltage Level section, specify the following parameters:



In the **Step 2: Pre-Signal Parameters** tab,

- **Level 1**: Type the initial voltage level.
- **Level 2**: Type the voltage to step to after the Level 1 voltage. The Level 1 voltage and the Level 2 voltage must differ by a minimum of 5 mV and a maximum of 100 mV.

  The **Pre-Signal Preview Graph** shows the waveform of the pre-signal voltage.

4. Do one of the following:

- Click **Save** to save the settings and close the Protocol Editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Click **Next** to edit other Channel Protocol settings.

Step 3: Specifying an Optional Conditioning Train

You can set up an optional voltage stimulus used to precondition the cell prior to applying the command voltage. Conditioning trains are inserted after the pre-signal. Cell currents are not recorded during the conditioning train, except for the last few milliseconds as needed to establish a signal baseline. The voltage is held at a level set to follow the train for a specified duration.

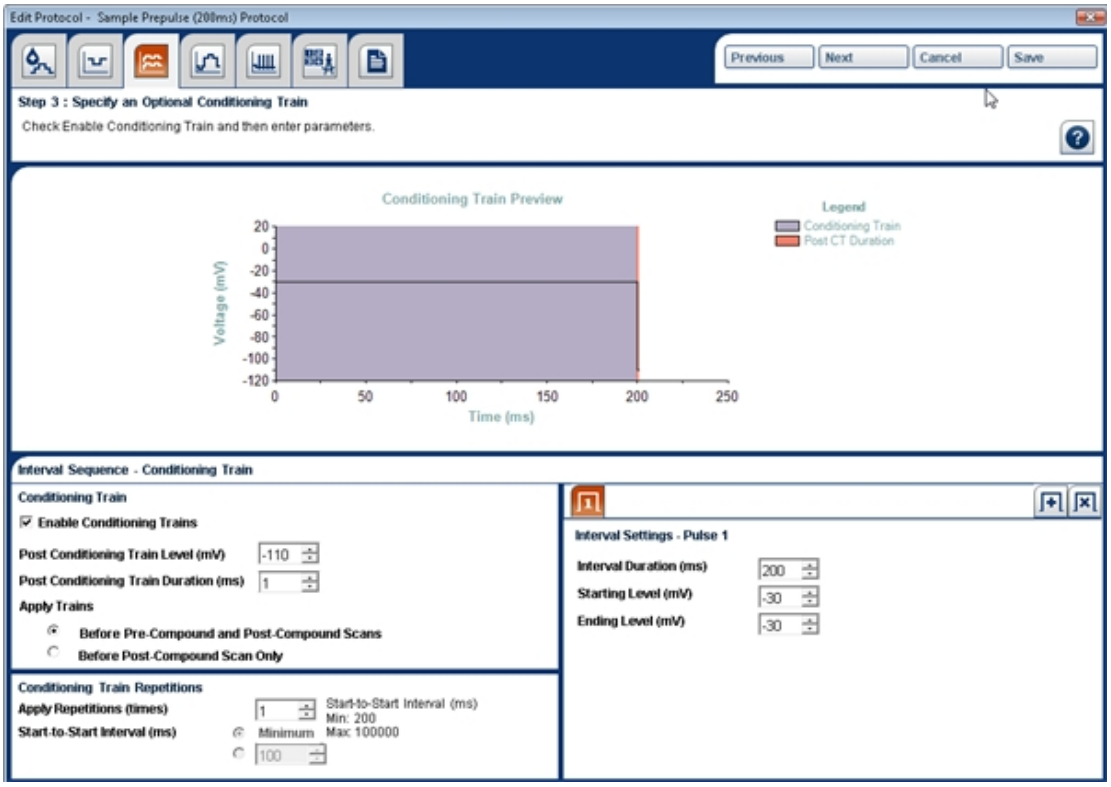
Configuration of the conditioning train is similar to that of the command voltage allowing a maximum of 10 intervals within the train, any sequence of which can be repeated. In addition, the entire conditioning train can be repeated. You can set the conditioning train to run before pre-compound addition scans and before post-compound addition scans, or only before the post-addition scan.

Note: No data is recorded during the conditioning train.

The **Conditional Train Preview** shows the waveform of the conditioning train voltage. As you specify the various parameters the graph updates to reflect your changes.

You can specify the following conditioning train parameters:

- Interval Sequence-Conditioning Train on page 77
- Conditioning Train Repetitions on page 78
- Interval Settings on page 79



Interval Sequence-Conditioning Train

The conditioning train can be configured with up to 10 intervals. Intervals can be defined as steps of constant voltage or voltage ramps.

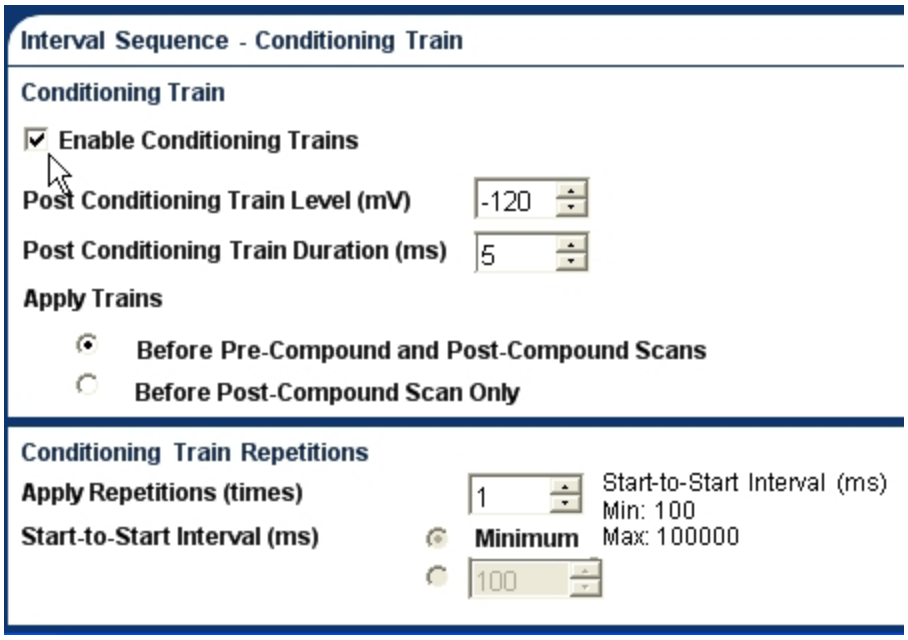
To specify the parameters for a conditioning train:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review

Workspace on page 15 for details.

2. Click **Edit** to open the Protocol Editor.
3. On the Step 3 tab in the Interval Sequence - Conditioning Train section, specify the following parameters:

- **Enable Conditioning Train**: Select the check box to enable the conditioning train configuration options. The conditioning train is an optional step.



- **Post Conditioning Train Level**: Type the voltage level to apply after the conditioning train and before the command voltage
- **Post Conditioning Train Duration**: Type the length of time the post conditioning train voltage level should be held before the command voltage is applied. The minimum value is 5 ms.
- **Apply Trains**: Select the option to specify when to run the conditioning train.

4. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Specify related parameters. For instructions, see:

  Conditioning Train Repetitions on page 78

  Interval Settings on page 79
- Click **Next** to edit other Channel Protocol settings.

Conditioning Train Repetitions

You can specify the number and duration of conditioning train repetitions.

To specify conditioning train repetitions:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. On the Step 3 tab in the Conditioning Train Repetitions section, specify the following parameters:

- **Enable Conditioning Train**: Select the check box to enable the conditioning train configuration options. The conditioning train is an optional step.
- **Apply Repetitions**: Type the number of times the conditioning train should repeat within the scan.
- **Minimum**: Specify that the conditioning train repetitions should start one right after the other with the minimum time between them. You can also choose the option to specify the **Start-to-Start Interval** in milliseconds. The interval you specify must be greater in length than the minimum interval.

4. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Specify Interval Settings on page 79
- Click **Next** to edit other Channel Protocol settings.

Interval Settings

You can specify the pulse for up to ten intervals in the waveform.

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit**.
3. On the Step 3 tab in the Conditioning Train Repetitions section, specify the following parameters:

- **Interval Duration**: Type the duration of the interval, up to 5000 milliseconds.
- **Starting Level**: Type the voltage to step to at the start of the interval. The range is –200 mV to +200 mV.
- **Ending Level**: Type the voltage to step to at the end of the interval. The range is –200 mV to +200 mV. If this voltage is the same as the **Starting Level** voltage then the single voltage is maintained for the duration of the interval as a voltage step. If it is different from the **Starting Level** then the voltage increases or decreases regularly throughout the interval to deliver a voltage ramp.

4. (Optional) Click  at far right of the section bar to add an interval pulse, and then specify parameters.

5. (Optional) Select an interval pulse tab to delete, and then click  at far right of the section bar.

6. Do one of the following:

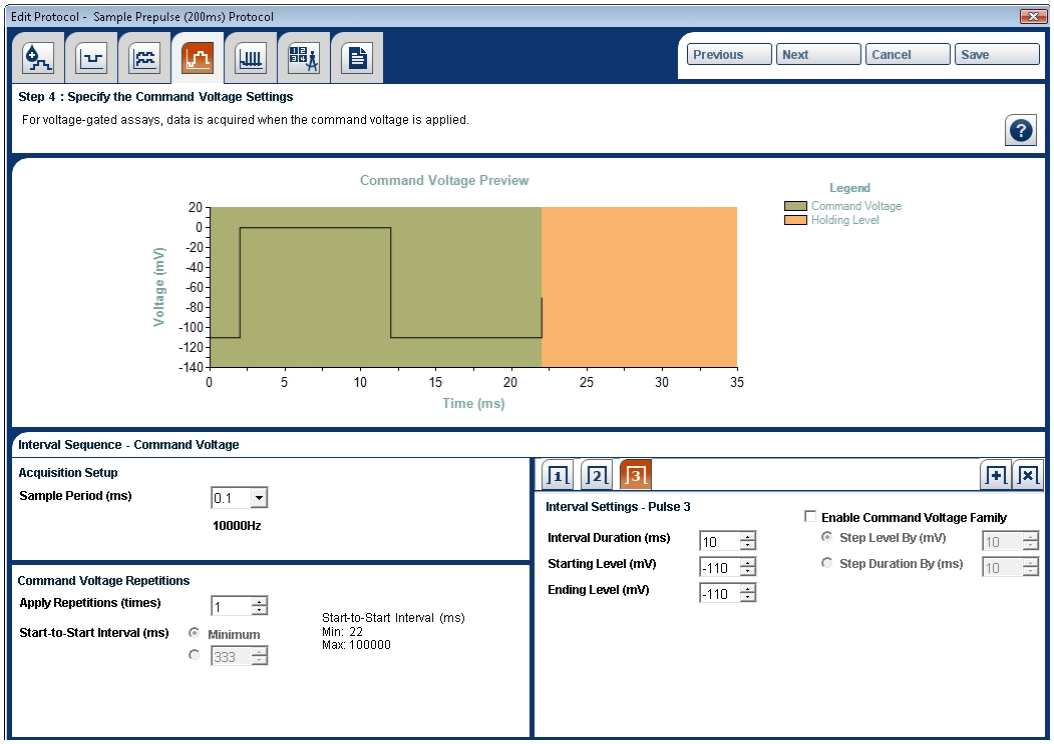
- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Click **Next** to edit other Channel Protocol settings.

Step 4: Specifying the Command Voltage

You can configure the sequence of voltage steps and ramps delivered to the PatchPlate wells.

The **Command Voltage Preview Graph**, under the Step 4 tab of the Channel Protocol editor, shows the waveform of the command voltage. As you specify the various parameters, this graph updates to reflect your changes.

Data acquisition for voltage-gated assays occurs only during delivery of the command voltage. You can configure a conditioning train to run prior to the command voltage. See Step 3: Specifying an Optional Conditioning Train on page 76.



The command voltage can each be configured with up to 10 "intervals." Intervals can be defined as steps of constant voltage or voltage ramps. You can also configure duration steps by stepping the interval time.

One step interval within the command voltage can have a "family" of voltages configured. This family of voltages is defined with a shift in the specified voltage in that interval for a given number of steps. Under these conditions, the command voltage is repeated as many times as there are family steps configured, with the command voltage changing in that interval as defined for each subsequent iteration of the sequence.

The entire command voltage can be configured to repeat a set number of times.

You can configure the following Command Voltage parameters:

- Interval Sequence Acquisition Setup on page 81
- Command Voltage Repetitions on page 82
- Interval Settings Command Voltage on page 82

Interval Sequence Acquisition Setup

You can specify the acquisition setup levels, repetitions and steps in the command voltage scan.

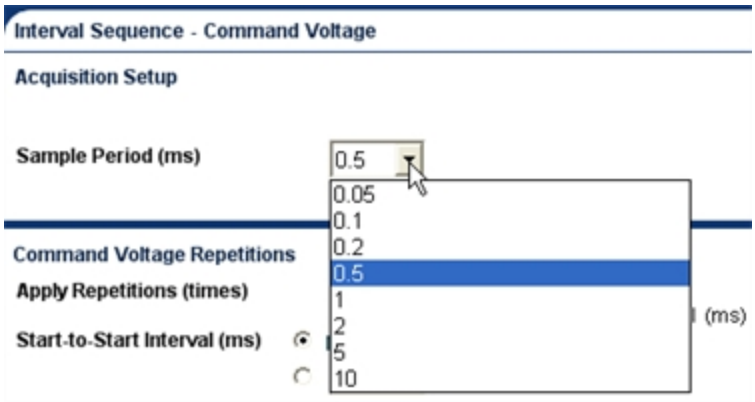


Figure 6-5: Sample Interval Sequence - Command Voltage Section

To set **Acquisition Setup** parameters:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. On the Step 4 tab in the Interval Sequence Command Voltage section, in the **Sample Period** field, type the length of time for the data sampling period in milliseconds per sample.
4. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Click **Next** to edit other Channel Protocol settings.

Command Voltage Repetitions

You can set the number of times the command voltage is applied during the sweep.

To set Command Voltage Repetition parameters:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. On the Step 4 tab in the Command Voltage Repetitions section, specify the following parameters:

- **Apply Repetitions**: Type the number of times the entire command voltage should repeat within the scan.
- **Minimum**: Select this to specify that the command voltage repetitions should start one right after the other with the minimum necessary time between them. You can also choose the option to specify the **Start-to-Start Interval** in milliseconds. The interval you specify must be greater in length than the minimum interval.

4. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Click **Next** to edit other Channel Protocol settings.

Interval Settings Command Voltage

You can specify up to ten pulses for one interval in wave form.

To set intervals for the command voltage:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit**.
3. On the Step 5 tab in the Interval Settings section, specify the following parameters:

- **Interval Duration**: Type the duration of the interval, up to 5000 milliseconds.
- **Starting Level**: Type the voltage to step to at the start of the interval. The range is –200 mV to +200 mV.

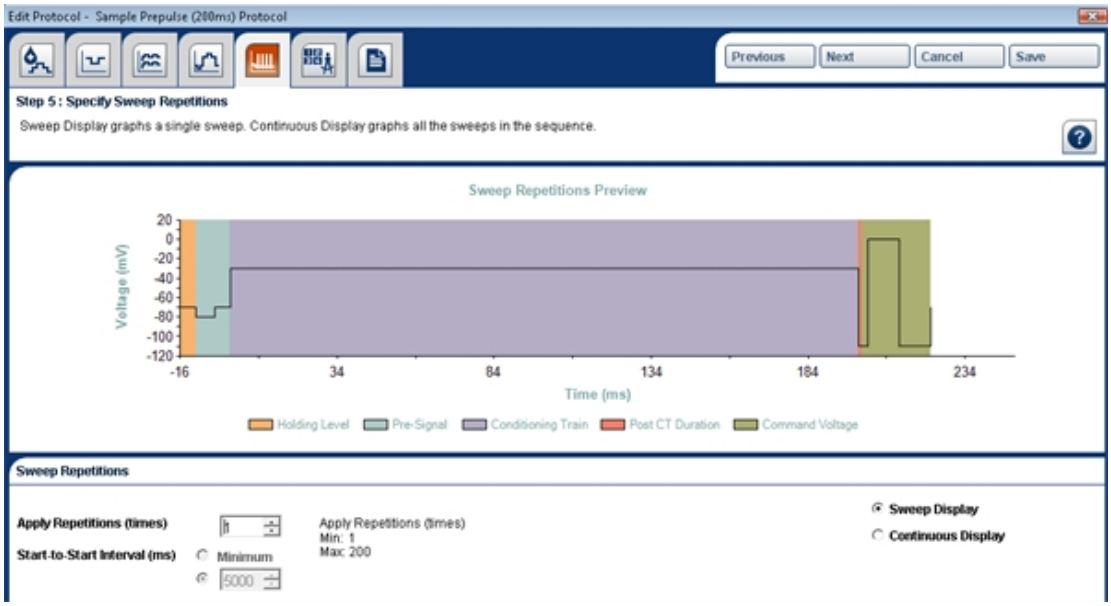
- **Ending Level**: Type the voltage to step to at the end of the interval. If this voltage is the same as the **Starting Level** voltage then the single voltage is maintained for the duration of the interval as a voltage step. If it is different from the **Starting Level** then the voltage increases or decreases regularly throughout the interval to deliver a voltage ramp.
- **Enable Command Voltage Family**: Select this check box to enable a command voltage family for one of the step intervals. This family of voltages is defined with a regular positive or negative shift in the specified voltage in that interval, for a given number of steps. Under these conditions, the command voltage is repeated as many times as there are family steps configured, with the command voltage changing in that interval as defined for each subsequent iteration of the sequence.
- **Step Level by (mV)**: Type the number of millivolts to change the interval with each repetition of the command voltage. To increase the interval, type a positive value in the **Step Level by (mV)** field. To decrease the interval, type a negative value in the **Step Level by (mV)** field.
- **Step Duration by (ms)**: Type the number of milliseconds to change the duration of each repetition of the command voltage.

4. (Optional) Click  at far right of the section bar to add an interval pulse, and then specify parameters.
5. (Optional) Select an interval pulse tab to delete, and then click  at far right of the section bar.
6. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Click **Next** to edit other Channel Protocol settings.

Step 5: Specifying Sweep Repetitions

In the **Step 5: Specify Sweep Repetitions** tab, the sweep repetitions preview graph shows the complete waveform specified in the previous steps including pre-signal, conditioning train, and command voltages. The entire configuration can be repeated for the number of times you specify with the Start-to-Start interval you define. In the Sweep Repetitions section, specify the number repetitions for the complete sweep including pre-signal, conditioning train, and command voltage.



To specify parameters for sweep repetitions, and to update the preview graph:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Protocol Editor.
3. On the Step 5 tab specify the following parameters:

- **Apply Repetitions**: Type the number of times to apply the entire sweep.
- Click the **Minimum** option to specify that the sweep repetitions should start one right after the other with the minimum necessary time between them. You can also choose the option to specify the **Start-to-Start Interval** in milliseconds. The interval you specify must be greater in length than the minimum interval.

4. Change the preview graph by selecting one of the following options:

- **Sweep Display** shows all the elements of a single sweep.
- **Continuous Display** shows all repetitions of the sweep in the entire scan. The preview graph changes in Continuous Display only if more than one repetition is specified.

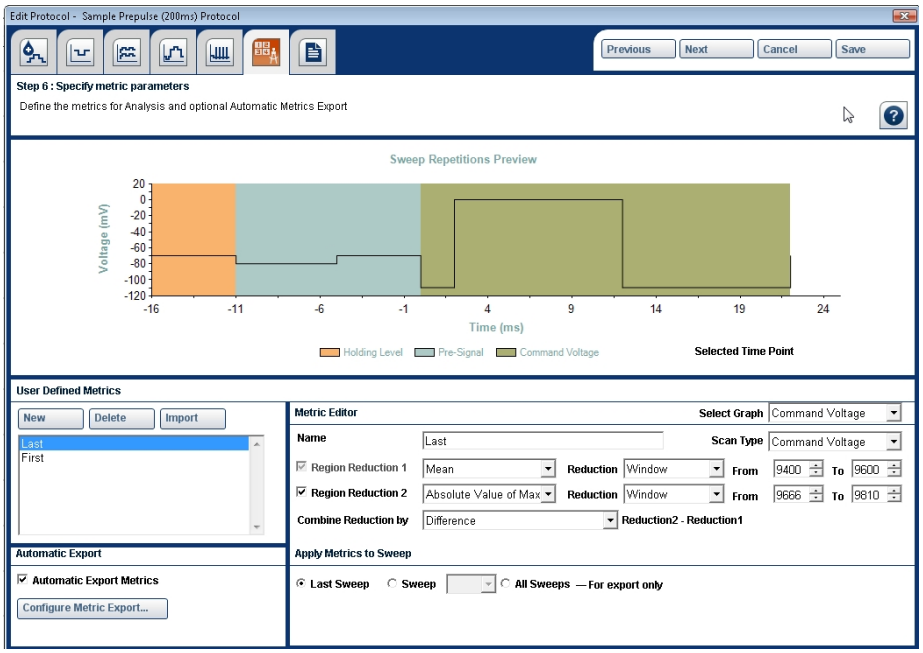
5. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Click **Next** to edit other Channel Protocol settings.

Step 6: Specifying Metrics Parameters

In the **Step 6: Specify Metrics Parameters** tab, you define the mathematical method for reducing the data acquired from each well. IonWorks Barracuda® System Software comes with pre-defined metrics for Offset and Resistance. In addition, you can define custom metrics as part of the Channel Protocol.

Using the Metrics Editor in the Channel Protocol Editor, or the Metrics Editor in the Data Analysis workspace, you can define simple or combined metrics. After they are defined, these metrics can be named, saved, and imported into other Channel Protocols.



Setting Options for the Metric Preview Graph

To set options for the Metric Preview Graph:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Channel Protocol Editor.
3. Go to the Step 6 tab and specify the following parameters:

- Select the type of graph to display in the preview area.
- Select **Command Voltage** for voltage-gated assays.
- Click in the graph to insert a labeled vertical marker as a time reference.

4. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Click **Next** to view Channel Protocol settings.

Creating a New Metric

User-defined metrics are named and saved with the Channel Protocol. If you define a new metric, it is named and saved with the Channel Protocol you are currently editing.

To view the parameters defined for any metric in the User Defined Metrics list, click the name of the metric. The fields in the Metric Editor display the selected metric settings.

Select a Graph as a reference for establishing the region of interest in each scan. Click in the graph to display the time value for the selected point.

To define a new metric:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit** to open the Channel Protocol Editor.
3. Go to the Step 6 tab in the Metric Editor section.
4. Name the metric settings by typing identifying text in the **Name** field. If you are importing a metric at a later time, this is the name you select to import the settings to your Channel Protocol.
5. Select a **Scan Type**:

- **Command Voltage** for voltage-gated assays.
- **Compound Addition** for ligand-gated assays.

6. In the **Region Reduction** field, click to define the method used to aggregate data. Define one or two reductions depending on the metric type, simple or combined. Select the 1st Reduction or 2nd Reduction option to view or edit each reduction. Reductions are applied to the selected region only.
7. In the **Reduction** field, define the selected time range for the calculated metric.

- Click **Entire Signal** for the entire duration of the sweep.
- Click **Window** to set a time range selected from the available data by typing values in the **From** and **To** fields.

8. In the **Combine Reduction** field, click a method to combine the values from the two reductions resulting in one output value. If defining two reductions, select a method to combine the values from the two reductions, resulting in one output value. Formulas used to calculate combined reductions are displayed when the method is selected.

Note: Reverse the selections in the **Reduction 1** and **Reduction 2** fields to invert the values in the formula.

9. Click the option to **Apply Metrics to Sweep**.

- **Last Sweep**: Applies the metric to the most recently acquired sweep data.
- **Sweep**: Sweeps are listed by sequence number. Select the number that corresponds to the sweep of interest.
- **All Sweeps-For Export Only**: Because a metric can be applied to only a single set of data, you cannot review multiple sweeps in the Data Analysis workspace. This option is available for exported data only.

10. Select the **Automatically Export Metric** check box to generate metric data files automatically upon completion of the Channel Protocol. For information on configuring automatically exported metrics, see Automatically Exporting Metrics Data on page 88.
11. Click **Save** to save the named metric settings with the Channel Protocol.
12. Do one of the following:

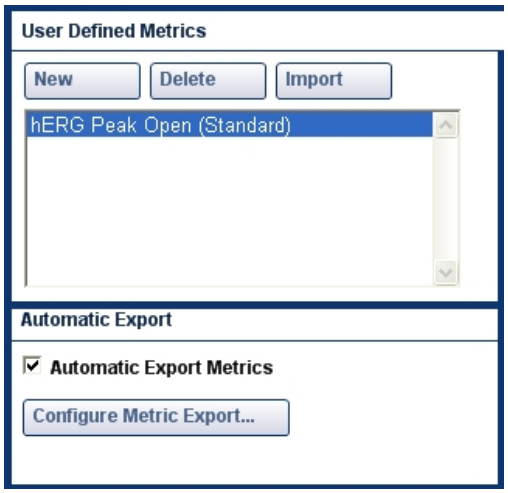
- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Click **Next** to view Channel Protocol settings.

Importing a Metric

Saved metrics from Channel Protocols can be imported into new Channel Protocols for reuse.

To import a saved metric setting file:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Channel Protocol from the **Channel Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.
2. Click **Edit**.
3. On the Step 6 tab in the User Defined Metrics section click **Import**.



4. In the User Defined Metrics section of the Metrics Editor, click **Import**.
5. Browse to the folder that contains the Channel Protocol with the metric you want to import.
6. Select the metric setting name from the file name list.
7. Click **Open**. The metric shows in the list of User Defined Metrics available.
8. Select the metric name in the list to display the metric settings in the Metric Editor fields.
9. If applicable, change the settings for the metric, and type a new name in the **Name** field.
10. Click **Save** to retain these metric settings with the current protocol.
11. Do one of the following:

- Click **Save** to save the settings and exit the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.
- Click **Next** to view other Channel Protocol settings.

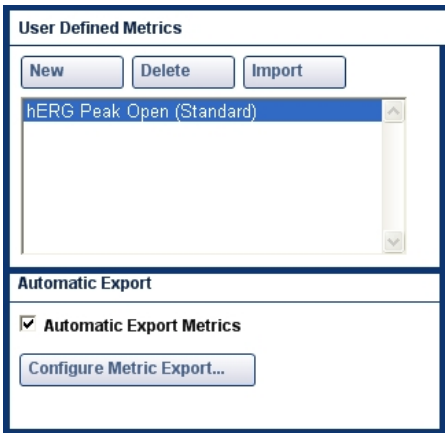
Automatically Exporting Metrics Data

Every Channel Protocol includes the option to automatically export data upon completion. Export methods set up to execute automatically at the completion of a Channel Protocol are not available for manual export. Manual export methods must be set up separately. For information on manually exporting data, see Exporting Metrics Data on page 143.

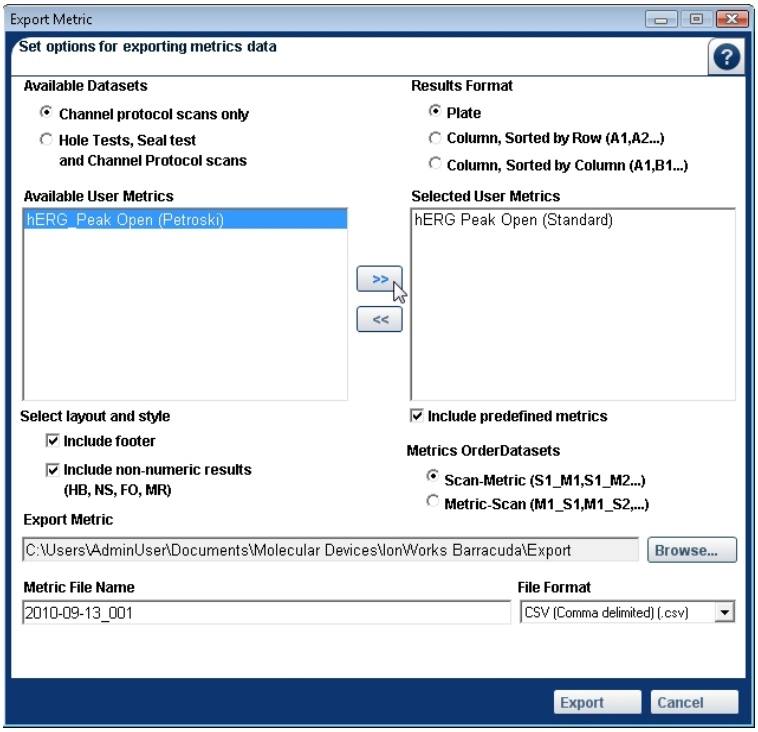
User-defined metrics set up to export automatically can be imported to other Channel Protocols, or used for manually exporting from a saved set of experiment data.

To automatically export metrics when the Channel Protocol completes:

1. Edit, import, or create your metric in the Channel Protocol Editor. For instructions, see Step 6: Specifying Metrics Parameters on page 85.



2. Check the box labeled **Automatic Export Metrics** at the conclusion of the Channel Protocol.
3. Click **Configure Metric Export** to open the Export Metric dialog.



4. Select the data set to include in the metric export: **Channel Protocol scans only** or **Hole Tests, and seal test and channel protocol scans**.
5. Select the **Results Format**:

- **Plate** format inserts results in a spreadsheet with a separate data set for each scan in a format that duplicates the well layout: rows A-P, and columns 1-24.
- **Column Sorted by Row** format lays out the results for each well in a descending order by row, A1, A2, A3... descending to P24, with scan results arranged in the columns from left to right across the spreadsheet.

- **Column Sorted by Column** format lays out the results for each well in a descending order by column, A1, B1, C1... descending to P24, with scan results arranged in columns from left to right across the spreadsheet.

6. Select the metric you named and defined from the list of **Available User Metrics**.
7. Click >> to move the selected metric to the **Selected User Metrics** field.
8. Check the boxes to include or exclude footer data. Footer data includes:

   A key to the column names for included scans, sweeps, and metrics.

   A key to non-numeric results: HB-hole blocked, NS-no seal, FO-filtered out, MR-manually rejected.

   Definitions for metric setting values and conditions.

   Protocol information including:

- Setup Data File Name
- Channel Data File Name
- Date/Time (local)
- Date/Time UTC
- Setup Protocol Name
- Channel Protocol Name

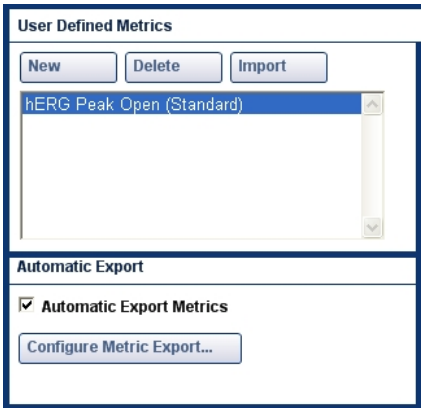
9. Check the box to include or exclude predefined metrics including basic voltage offset values and resistance values.
10. Select the **Metrics Order Dataset** by Scan-then-Metric values (S1_M1, S1_M2, S1_M3...) or Metric-then-Scan values (M1_S1, M2_S1, M3_S1...).
11. If needed, browse to specify the path to the folder for your exported metrics data in the **Export Metric** field. The default path setting is **C:\Users\AdminUser\Documents\Molecular Devices\IonWorks Barracuda\Export**.

    Note: If you have an invalid **Export Metric** path entered, such as to a USB flash drive that has been removed, the software automatically exports the data to the default location **C:\Users\AdminUser\Documents\Molecular Devices\IonWorks Barracuda\Export**.
12. Type a name for the exported metric data file in the **Metric File Name** field. IonWorks Barracuda® System Software automatically creates a file for the exported metric data named with the experiment-name appended with the metric file name you type.
13. Select a file format from the **File Format** field: CSV for comma-delimited data, or TXT for tab-delimited data.
14. Click **OK**. The data exports immediately to your selected folder.
15. To view the exported data, click **File > Open Exported Data Folder** and select from the list of exported data files.

Deleting a User-Defined Metric

To delete a user-defined metric from a Channel Protocol:

1. Select the Channel Protocol that includes the saved metric from the Channel Protocols field.
2. Click **Edit** to open the Channel Protocol Editor.
3. Select the tab for **Step 6: Specify Metrics Parameters**.



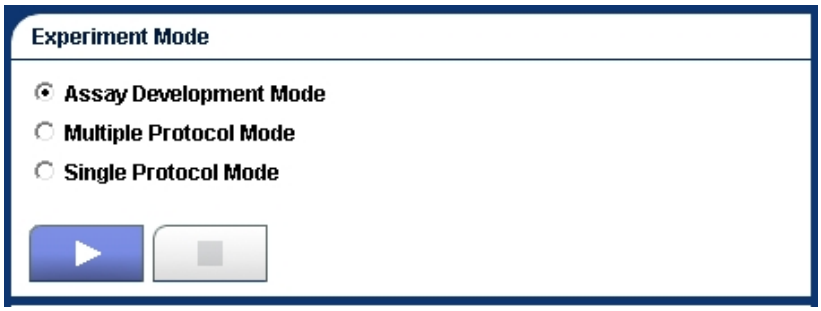
4. Click the name to select the metric you want to delete.
5. Click **Delete**. The metric settings are removed from the Channel Protocol you are editing.

Modifying a Channel Protocol in Assay Development Mode

When you want to optimize a new type of assay, you can open a pre-defined Channel Protocol from Molecular Devices, and then optimize the sample assay using Assay Development Mode.

To modify a pre-defined Channel Protocol in Assay Development Mode:

1. Select a **Setup Protocol** from the list of saved protocols.
2. Select a **Cleanup Protocol** from the list of saved protocols.
3. From the list of pre-defined Channel Protocols, select the **Channel Protocol** that most closely matches the assay for which you would like to optimize.
4. To preview the parameters for the pre-defined protocol, click **View**.
5. To make changes before running the Channel Protocol, click **Edit**. The **Edit Channel Protocol** dialog appears.
6. Make any necessary changes to the parameters listed for the Channel Protocol.
7. If the **Save Protocol As Read Only** check box is selected, change the **Protocol Name**, then click **Save**. If the **Save Protocol As Read Only** check box is not selected, click **Save**. The **Edit Channel Protocol** dialog closes.
8. In the Data Acquisition and Review workspace, in the **Experiment Mode** section, select **Assay Development Mode**.

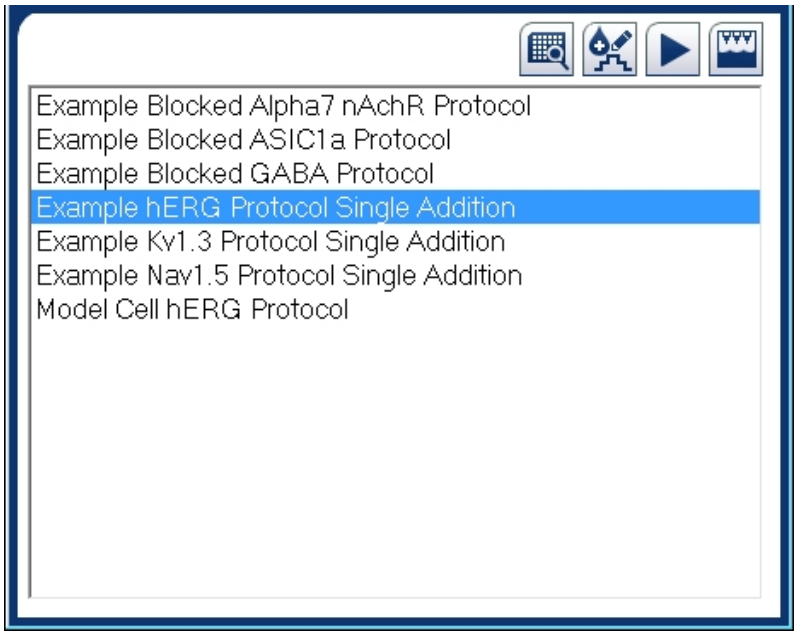


9. Click  to start the assay.

   The **Add Experiment Notes** dialog appears.

10. Type your experiment notes and then click **Continue**. See Adding and Viewing Experiment Notes on page 121.

    The instrument runs through the Setup Protocol and the Channel Protocol and then pauses. The **Assay Development** dialog appears.



11. Click  to review Channel Protocol data in the Data Acquisition and Review workspace. The plate view, **Fixed Well View**, and **Floating Well View** functions are all active for your data review.

12. Click  to return to the **Assay Development** dialog.

13. Choose how to continue. Choices include: **Edit selected Channel protocol**, **Run protocol**, or **Run cleanup protocol**, which finishes the Assay Development process.

    - To edit a Channel Protocol, select the protocol name from the list, and click  to open the **Edit Channel Protocol** dialog. Continue with step 16.

- To run a Channel Protocol, select the protocol name from the list, and click  to run it. When the **Assay Development** dialog returns, repeat steps 13 to 15.
- To finish Assay Development, click  . The selected Cleanup Protocol runs to end the experiment.

14. Make any adjustments to the parameters, and then click **Save**.
15. If the protocol you edited was marked **Read-Only**, then type a new **Protocol Name** and click **Save** to return to the **Assay Development** dialog.
16. Click  to run the edited Channel Protocol. When the **Assay Development** dialog returns, repeat steps 14 to 16.

Creating Multiple Channel Protocols

Before creating a Multiple Channel Protocol, you must have several single Channel Protocols. You can use the example protocols provided by Molecular Devices or create your own. See Creating a Channel Protocol on page 67.

In the Data Acquisition and Review workspace, in the **Protocol** section, when selected in the **Experiment Mode**, the **Channel Protocol** field changes to the **Multiple Channel Protocol** field. The **Multiple Channel Protocol** field lists saved routines composed of a series of **Channel Protocols** set to run consecutively.

The **Selected Channel Protocols** routine that you create in the **Edit Multiple Channel Protocol** dialog can be saved for selection in the **Multiple Channel Protocol** field for repeated future usage. When you run an experiment in **Multiple Protocol Mode**, you select a saved **Multiple Channel Protocol**, along with a **Setup Protocol** and a **Cleanup Protocol**.

To create a Multiple Channel Protocol:

1. Start an experiment in **Multiple Protocol Mode**. For instructions, see Running an Experiment on page 117.
2. Enter a name for the Multiple Channel Protocol you are creating in the **Protocol Name** field.
3. Enter a description of this Multiple Channel Protocol in the **Protocol Description** field.
4. Check **Read Only** if you want to write-protect this Multiple Channel Protocol after it is saved.
5. Specify the number of seconds to pause before continuing with the next Channel Protocol in the **Delay Between Protocols** field.
6. (Optional) Edit a Channel Protocol from the **Available Channel Protocols** list. See Editing a Channel Protocol in the Multiple Channel Protocol Editor on page 95.

7. Select a Channel Protocols to run in the experiment from the **Available Channel Protocols** list, and use the right arrow button to move the selected Available Channel Protocol to the **Selected Channel Protocols** list. The moved protocol appears last in the list.
8. Repeat steps 6 and 7 as needed.

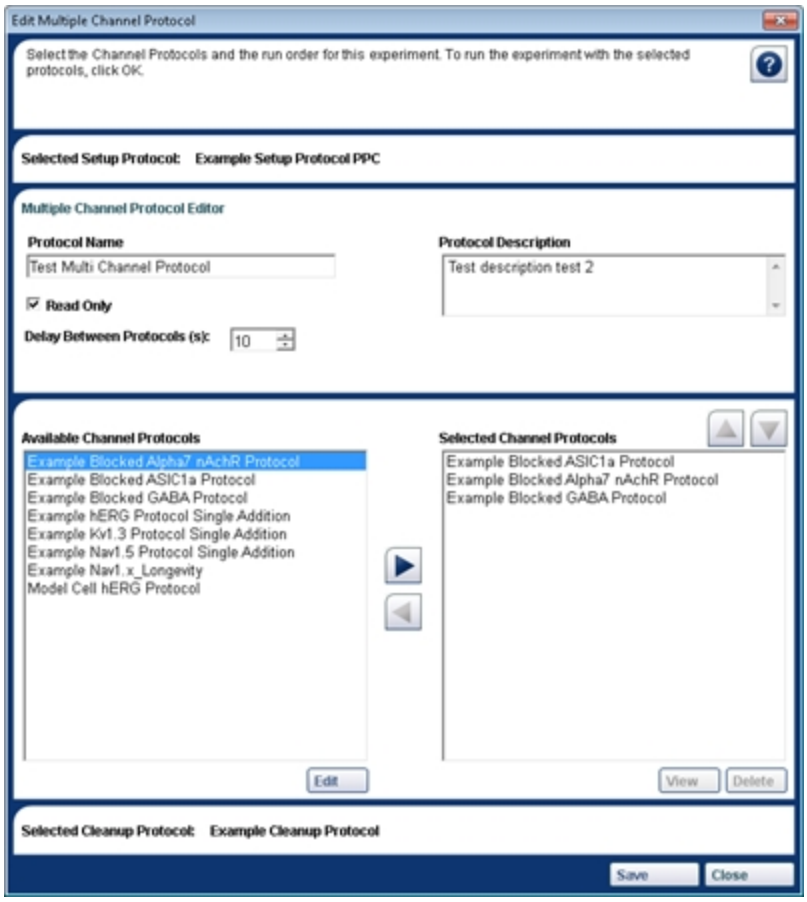


Figure 6-6: Edit Multiple Channel Protocol dialog

9. Use the up and down arrow buttons to reorder the **Selected Channel Protocols** list.
10. When you are finished creating your Multiple Channel Protocol, click **Save**. The **Edit Multiple Channel Protocol** dialog closes.

Editing a Channel Protocol in the Multiple Channel Protocol Editor

As you develop a Multiple Channel Protocol, if needed, you can edit an existing Channel Protocol from within the **Edit Multiple Channel Protocol** dialog. The Channel Protocols in the **Selected Channel Protocols** list are copies of the protocols from the **Available Channel Protocols** list at the time they were added to the **Selected Channel Protocols** list. You can only edit a Channel Protocol in the **Available Channel Protocols** list. Edits to a protocol in the **Available Channel Protocols** list are independent of the copy in the **Selected Channel Protocols** list.

Note: If you edit a protocol in the **Available Channel Protocols** list without changing the protocol name, and move the edited protocol to the **Selected Channel Protocols** list, the edits will automatically apply to all of the **Selected Channel Protocols** with the same name.

To edit a Channel protocol in the Multiple Channel Protocol editor:

1. Select a Channel Protocol in the **Available Channel Protocols** list, and click **Edit**.
2. When the **Edit Channel Protocol** dialog appears, review the parameters in each of the protocol editor tab steps, and change parameters as applicable for the current experiment.
3. Click **Save** to finish. If **Save Protocol As Read-Only** has been selected, change the **Protocol Name** and then click **Save**.
4. When the **Edit Multiple Channel Protocol** dialog appears, you can select the edited protocol, and move it, using the right arrow button, from the **Available Channel Protocols** list to the **Selected Channel Protocols** list. The moved protocol appears last in the list.

   Note: When moving the edited protocol, if the protocol name is the same as a protocol already in the **Selected Channel Protocols** list, you will be prompted about whether you want the edited protocol to overwrite the existing protocol with the same name.

5. Continue with step 7 in Creating Multiple Channel Protocols on page 93.

Deleting a Channel Protocol in the Multiple Channel Protocol Editor

As you develop a Multiple Channel Protocol from within the **Edit Multiple Channel Protocol** dialog, if needed, you can delete a Channel Protocol that was moved to the **Selected Channel Protocols** list.

Note: Channel Protocols in the **Available Channel Protocols** list cannot be deleted.

To delete a Channel Protocol in the Multiple Channel Protocol editor:

1. Select a Channel Protocol in the **Selected Channel Protocols** list, and click **Delete**.
2. Continue with Creating Multiple Channel Protocols on page 93.

Chapter 7: Creating a Cleanup Protocol

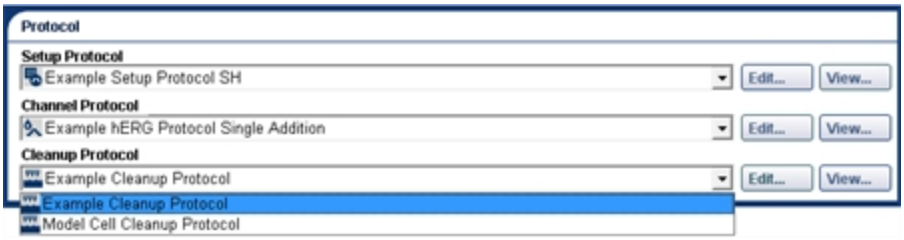
7

The Cleanup Protocol automates necessary maintenance steps between assays such as washing the pipettor tips, washing the CellPettor, and washing the electrodes on the Electrode-Plate.

For wash solution options, see the List of Approved Fluids on page 187.

To create a Cleanup Protocol:

1. In the Protocol section of the Data Acquisition and Review workspace, select a Cleanup Protocol from the **Cleanup Protocol** selection field. See Data Acquisition and Review Workspace on page 15 for details.



2. Click **Edit** to open the Protocol Editor.
3. Review the parameters in the protocol editor. See Step 1: Specify Instrument Cleanup on page 97.
4. When you have finished setting the parameters, click **Save** to save your changes and close the protocol editor. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.

Step 1: Specify Instrument Cleanup

In the **Step 1: Specify Instrument Cleanup** tab, set values for the type of solution and the number of repetitions in the wash function.

- The CellPettor is always washed after use.
- The Plenum is always drained at the appropriate time.

To specify instrument cleanup parameters:

1. Select the **Wash plenum** check box, and specify settings.
   - In the **Soak Duration** field, type the soak time in minutes (maximum 1440 minutes/24 hours).
   - In the **Number of cycles** field, type the number of times the soak cycle repeats (maximum 5).
2. Select from the two **Wash Tip Options**.

- Click **Wash Tips and Electrode-Plate** to keep the tips on the pipettors, and wash both the Electrode-Plate, and the tips.
- Click **Discard Tips** to eject the tips from the pipettors after the Cleanup Protocol finishes. Tips can be unloaded from the pipettor head and reloaded approximately nine times before you must replace the tip rack with new tips.

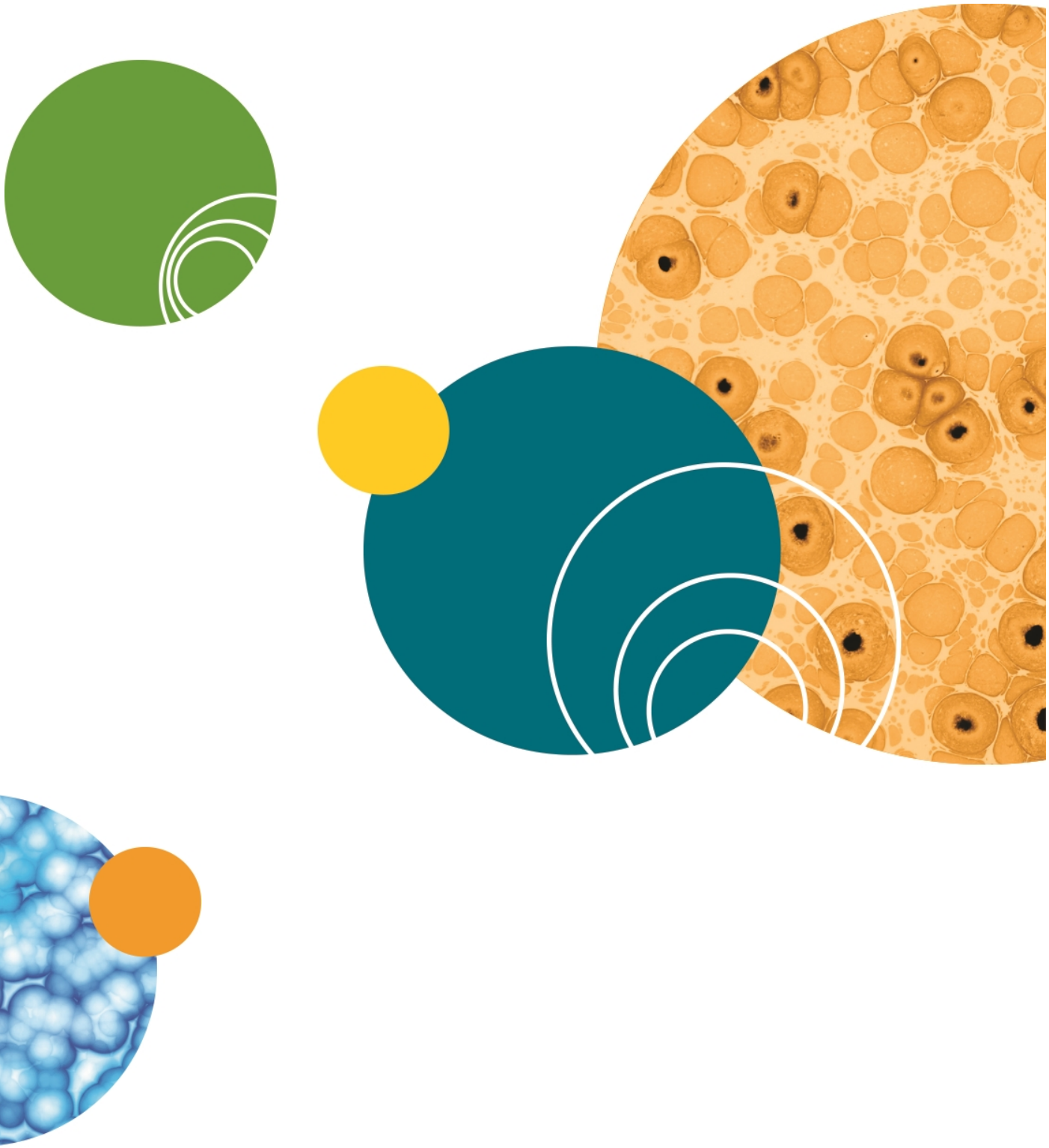
3. Specify **Tips Washing** parameters.

- Click the **Wash Source** list and select the fluid source (A or B) for the first wash cycle.
- Select the **Enable Ultrasound Cleaning** check box to sonicate during the cleaning operation.

WARNING! Do not enable ultrasonic cleaning while using any flammable liquid such as alcohol (EtOH) in the Source A or Source B bottles. Sonication can cause flammable liquids to evaporate into the enclosure, posing a potential risk of fire or explosion.

- Click **Wash Fluid 2** to specify a second wash operation.
- Click the **Wash Source** list and select the fluid source (A or B) for this wash cycle.
- In the **Number of Strokes per Wash Cycle** field, type the number of times the tip will be dipped into the wash fluid in one wash cycle (range 1 to 20).
- In the **Number of Wash Cycles** field, type the number of times the entire wash cycle is repeated. The wash station is filled and drained for each separate wash cycle.

4. Specify **Electrode-Plate** parameters. **Wash Fluid 1** cannot be disabled.
   - Click the **Wash Source** list and select the fluid source (A or B) for the first wash cycle.
   - Click **Enable Ultrasound Cleaning** to sonicate during the cleaning operation.
     Do not enable ultrasonic cleaning while using any flammable liquid such as alcohol (EtOH) in the Source A or Source B bottles. Sonication can cause flammable liquids to evaporate into the enclosure, posing a potential risk of fire or explosion.
   - Click **Wash Fluid 2** to specify a second wash operation.
   - Click the **Wash Source** list and select the fluid source (A or B) for this wash cycle.
   - In the **Dwell Time Per Wash Cycle** field, type the number of minutes to suspend the Electrode-Plate in the wash fluid.
   - In the **Number of Wash Cycles** field, type the number of times the entire wash cycle is repeated. The wash station is filled and drained for each separate wash cycle.
5. Review the parameters in each of the protocol editor steps, changing them as applicable for the current experiment.
6. Click **Save**. If the protocol is marked Read-Only, type a new name for the protocol, and then click **Save**.



Chapter 8: Preparing the Instrument for an Experiment Run

8

After you have turned on the system computer and turned on the instrument, a number of maintenance procedures must be followed to prepare the instrument for use.

To prepare the instrument for an experiment:

1. Empty all waste carboys.
2. Prepare the system fluids.
   - Fill the Source A and Source B carboys with wash solution.
   - Fill the internal buffer, external buffer, alcohol, and cell perforation agent bottles.
3. Run the Start of Day Flush and Rinse procedure to pump out alcohol from the system and prime with assay solutions.
4. Load the required labware including a filled external buffer boat, PatchPlate, pipettor tips (if not already loaded),
5. Prepare compound plates, if applicable, and load them at the compound stations.
6. Load a pipette into the CellPettor.
7. Prepare cell suspension, remove the cells to a conical tube, and load the tube into the cell tube holder on the process deck.

Preparing the Fluid-Handling System

The fluid-handling system delivers source fluids to the process deck and pumps out waste fluids.

The most important steps in maintaining the fluid-handling system are:

- Performing the End of Day Flush and Rinse on page 149 to sterilize the system by flushing out saline solutions and replacing them with alcohol (50% EtOH).
- Performing the Start of Day Flush and Rinse on page 105 to flush out the alcohol (50% EtOH) and primes the system with saline solutions for the day's experiments

To prepare the instrument for use, you must empty the waste carboys, and fill each of the source fluid bottles with the minimum working volume.

Fluids include:

- **External buffer** (extracellular) is pipetted into the PatchPlate wells before the hole test is conducted in the Setup Protocol. External buffer is also used as a rinse solution for the CellPettor and is generally used in preparing compounds.
- **Internal buffer** (intracellular) fills the plenum below the PatchPlate. Internal buffer is also generally used in preparing the cell perforation agent solution.

- **Cell perforation agent** is most commonly a diluted solution of Amphotericin B and internal buffer.
- **Wash solutions or solvents** are used during protocols to rinse the pipettor tips and the electrodes between experiments.
- **Alcohol (50% EtOH)** is used for the End of Day Flush and Rinse procedure to sterilize the equipment.

CAUTION! Do not use bleach on the surfaces of the instrument, or in the fluid bottles or lines unless specified by a Molecular Devices procedure. Bleach can cause discoloration of bottles, lines, and surfaces, and can cause damage that leads to equipment failure.

Emptying Waste Carboys

Emptying the waste carboys is a simple, but critical task in preparing the instrument for daily use. Two types of lines run from the carboy caps to the cabinet frame: fluid tubes and overflow sensor wires. The lines must be disconnected from the cabinet frame using the quick-connectors before you lift out the carboys.

Three waste carboys below the process deck must be emptied daily to prevent overflow:

- Waste A
- Waste B
- Plenum/Cell Waste

WARNING! Always wear protective equipment and review all applicable laboratory site regulations when handling cell solutions and cell waste. Always follow the appropriate procedures for the biosafety level of the substance you are handling.

To empty the carboys:

1. Locate the waste carboys in the fluidics drawer in the lower compartment of the instrument cabinet. (For a diagram, see The Fluid Handling Components on page 40.)
2. Disconnect the fluid lines by pushing the connector down to activate the quick-release mechanism.
3. For the sensor line, push the metal tab on the connector to activate the quick-release mechanism.
4. Remove the carboys and unscrew the caps.
5. Dispose of the solutions as applicable. When disposing of cell waste, follow your company procedures for handling biohazardous material.
6. Rinse the bottles as applicable.

7. Replace the caps, and then return the carboys to their correct positions in the holding drawer.
8. Reconnect the sensor and fluid lines by pushing each connector into its corresponding connection joint until it clicks firmly into place. Make sure the fluid lines are properly connected. If the fluid connections are not properly engaged, fluid lines cannot function and the instrument enters a fault state.

Note: Two temporary waste bottles are used to capture small volumes prior to pumping to the larger waste carboys. These temporary waste bottles do not require maintenance and do not need to be hand-emptied.

Filling Buffer, Alcohol, and Cell Perforation Agent Bottles

Calculate the minimum fill volume for each source bottle by multiplying the number of intended experiments by the volume used per experiment. Remember to add the dead volume for each bottle:

Source Fluid	Volume Used Per Experiment	Dead Volume
Internal buffer	50 mL	30 mL for 500 mL bottle
External buffer	30 mL	60 mL for 1000 mL bottle
Cell perforation agent	38 mL	15 mL for 250 mL bottle

When preparing for an experiment run, make sure you also empty the waste carboys and fill source bottles with wash solution.

To fill the internal and external buffer bottles, cell perforation agent bottle, and the alcohol bottle:

1. Open the lower cabinet to locate the external buffer bottle and the alcohol bottle on the upper rack. Locate the internal buffer bottle and the cell perforation agent bottle on the rack beneath the PatchPlate station.
2. To disconnect each of the lines from the caps, push on the metal connector-joint tab to activate the quick-release mechanism.
3. Lift the bottles from their racks and remove the caps.
4. Rinse source bottles thoroughly with de-ionized water.
5. Fill the internal buffer source bottle with internal buffer. Adjust the final volume based on the total number of intended experiment runs. Use the internal buffer solution appropriate to the cell line or ion-channel you are recording.
6. Fill the cell perforation agent bottle with 100 µg/mL Amphotericin B in internal buffer. (See Preparing Cell Perforation Agent on page 105.) Adjust the final volume based on the total number of intended experiment runs.
7. Fill the external buffer bottle with the external buffer of choice. Adjust the final volume based on the total number of intended experiment runs.

The recommended external buffer is a balanced salts solution such as Dulbecco's Phosphate Buffered Saline solution 1X with calcium and magnesium filtered with a 0.1 µm or 0.2 µm filter. External solutions used in conventional patch clamp electrophysiology can also be used on the IonWorks Barracuda System, such as HEPES-buffered balanced salts solution.

8. Fill the alcohol bottle with at least 100 mL of Ethyl Alcohol. Use reagent-grade Sigma-Aldrich (Cat. R8382) diluted to a maximum 50% concentration.
9. Replace the caps and return the bottles to their correct positions on the holding rack.
10. Reconnect the fluid lines on each of the bottles by pushing the connector into the cap until the metal tab clicks firmly into place.
11. Close the lower cabinet.

Filling Source Bottles with Wash Solutions

Source A and Source B carboys reside in the fluidics drawer behind the Waste A and Waste B carboys. The caps face toward the rear of the instrument. These six-liter carboys hold wash solutions or solvents used to wash pipette tips and the Electrode-Plate electrodes. You can use two different wash solutions or solvents in each carboy, or you can fill them both with the same fluid. See the List of Approved Fluids on page 187 to determine whether your wash solution is acceptable.

CAUTION! Do not use bleach on the surfaces of the instrument, or in the fluid bottles or lines unless specified by a Molecular Devices procedure. Bleach can cause discoloration of bottles, lines, and surfaces, and can cause damage that leads to equipment failure.

To fill the source bottles:

1. Locate the source bottles in the lower compartment of the Barracuda cabinet. Pull out the holding rack to access the source bottles located in the rear of the drawer.
2. Disconnect the fluid lines by pushing on the metal connector-joint tab to activate the quick-release mechanism. Remove the bottles from the rack and remove the caps.
3. If applicable, rinse source bottles thoroughly with clean, de-ionized water.
4. Fill Source A and Source B bottles with your preferred wash solution.

   The recommended wash solution is Ca/Mg-free external buffer of choice or Ca/Mg-Free Dulbecco's Phosphate Buffered Saline 1X solution filtered using a 0.1 µm or 0.2 µm filter.
5. Replace the caps and return the source bottles to their correct positions in the holding rack.
6. Reconnect each fluid line by pushing in the connector until it clicks firmly into place. Make sure fluid lines are properly connected.

Preparing Cell Perforation Agent

Cell perforation solution fills the plenum reservoir below the PatchPlate when **Add Perforation Agent** is specified as a step in the Obtain Cell Access section of the Setup Protocol Editor.

Amphotericin B (Sigma-Aldrich P/N A4888) is the recommended cell perforation agent for use in the IonWorks Barracuda System. General guidelines are 100 µg of cell perforation agent for each milliliter of internal buffer solution. Prepare the cell perforation solution according to your lab guidelines, or follow these instructions.

To prepare Amphotericin B solution:

1. Dissolve 10 mg Amphotericin B in 360 µL DMSO.
2. Sonicate for five minutes. The solution must be uniformly clear without any precipitates.
3. Add the dissolved Amphotericin solution to 100 mL of internal buffer and vortex for one minute. Do not filter.

Note: Amphotericin is light sensitive and degrades over time. Prepare fresh Amphotericin for each series of runs to avoid degradation.

Performing the Start of Day Flush and Rinse

The Start of Day Flush and Rinse is a mandatory daily maintenance procedure that must be run before any experiments are performed.

The Start of Day Flush and Rinse, initializes the system, flushes out the alcohol (EtOH) from the End of Day Flush and Rinse and primes the system with assay solutions for the day's experiment.

Before running the Start of Day Flush and Rinse procedure, the waste carboys must be emptied and the source fluid bottles in the system must be filled. See Preparing the Fluid-Handling System on page 101.

To perform the Start of Day Flush and Rinse:

1. Make sure waste carboys are empty and source bottles are filled with sufficient volumes for the number of planned experiments. The Start of Day Flush and Rinse uses approximately:
   - 65 mL of internal buffer
   - 20 mL of external buffer
   - 6 mL of cell perforation agent
2. If not on the process deck from the last usage, place a clean, undamaged PatchPlate into the PatchPlate nest, load a CellPettor pipette, and close the instrument door.

CAUTION: A clean PatchPlate can be reused as long as it is in good condition with no damage to any part of the consumable. Using a delaminated, chipped, marred, or misshapen PatchPlate could result in damage to the Electrode-Plate, or instrument errors.

3. In the shortcut bar, click  , and then click **Start of Day Flush and Rinse**.
4. Click **Continue**. The **Start of Day Flush and Rinse** process runs for approximately five minutes, and includes initializing the system and priming the fluid lines.
5. Remove the PatchPlate when the process stops and the instrument door unlocks.

Preparing the Process Deck

Before starting an experiment, the necessary plates or boats must occupy the correct stations of the process deck. Each station has a sensor that detects the presence of a plate or boat. If the process deck is not properly prepared, the instrument responds with an error and the experiment cannot be completed.

Loading the Tip Rack

The tip rack station holds a tray pre-loaded with 384 disposable pipettor tips. Molecular Devices black tips [PN 5011976] or clear tips [PN 5011975] are approved for use with the Barracuda instrument. Black tips are recommended.

To reuse the tips, you can specify a tip washing step in the Tip Washing section of the Cleanup Protocol Editor.

Note: Tips can be unloaded from the pipettor head and reloaded approximately nine times before you must replace the tip rack with new tips.

To load the tip rack onto the process deck:

1. Unwrap a new set of disposable pipettor tips and remove the rack lid.
2. Open the glass door to the top of the instrument cabinet and locate the Tip Rack station.
3. Push back the spring-loaded indexer at the right rear corner of the tip rack nest.
4. Slide the tip rack into the plate nest and release the indexer.
5. In the shortcut bar, click  , and then click **Load Tips**.

Loading the PatchPlate

A clean, uncontaminated PatchPlate must be used for each experiment. Make sure you specify the correct **PatchPlate Type** in the Setup Protocol. See Selecting the PatchPlate Type on page 57.

CAUTION! Make sure the PatchPlate is correctly registered at the left edge and the front edge of the plenum nest with the A1 corner fitted against the plenum. Make sure the PatchPlate is properly seated on all sides against the plenum base. Failure to correctly insert the PatchPlate can lead to extensive damage to the Electrode-Plate.

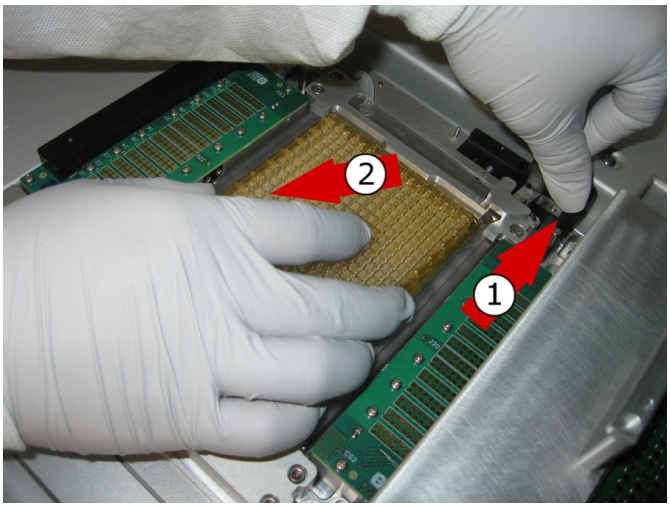
CAUTION! Wear gloves when handling PatchPlates. To prevent contamination or damage, always avoid touching the membrane on the bottom of the PatchPlate.

To load the PatchPlate:

1. Make sure the plenum is dry before loading a new PatchPlate. Avoid the cable on the left side of the plenum and gently blot the plenum with a Kimwipes® Delicate Task Wiper or a WYPALL® Wiper. See Plenum on page 34.

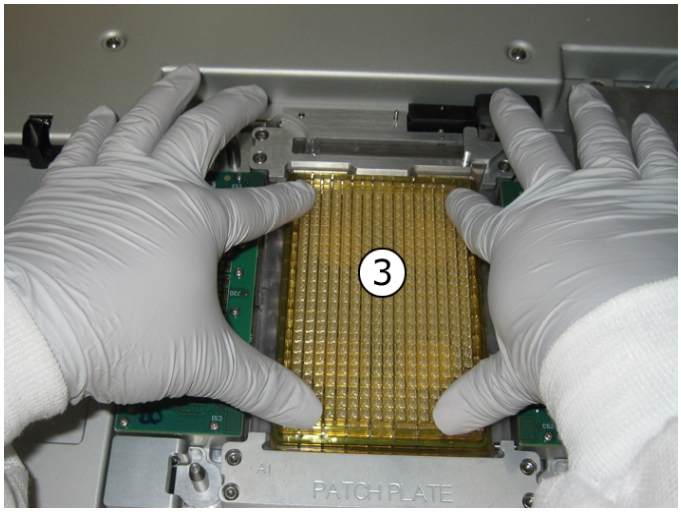
   Do not touch the round, ground electrodes on the surface of the plenum. Doing so could result in a build-up of oils on the electrodes. Oil buildup on the electrodes impedes their functionality.

2. Unwrap a new PatchPlate without touching the membrane on the bottom of the plate.
3. Position the PatchPlate on the plenum with the **A1** well at the front-left corner.
4. Hold back the spring-loaded indexer at the right-rear corner of the plenum nest. (Figure arrow 1.)



5. Slide the PatchPlate into the nest with the left flange of the plate against the edge and the **A1** corner fitted against the plenum. (Figure arrow 2.)
6. Make sure the left edge of the PatchPlate is fully registered against the left edge of the plenum.
7. Make sure the front edge (closest to you) of the PatchPlate is fully registered against the front edge of the plenum.

8. Make sure the PatchPlate is properly seated against the plenum base. Gently press all edges down to ensure the PatchPlate is lying flat without any lifting on any side. (Figure 3.)



9. Release the spring-loaded indexer. A light tap on the indexer after release helps to ensure the PatchPlate is securely fitted at the A1 corner.

Reloading the PatchPlate after an Error

If an error message appears because the PatchPlate is not correctly positioned:

1. Remove the Electrode-Plate from the plenum and move it to the wash station.
2. Remove the PatchPlate from the plenum nest.
3. Re-insert the PatchPlate using the previous procedure (Loading the PatchPlate on page 106).
4. In the shortcut bar, click  , and then click **Initialize**.
5. When the initialization process completes, continue with your experiment.

Loading and Filling the External Buffer Boat

Load a clean, dry external buffer boat into position in the external buffer station of the process deck, and then fill the boat with buffer solution before the start of an experiment.

Note: The minimum fill volume for the boat is 25 mL. The maximum volume is 125 mL.

The recommended external buffer is a balanced salts solution such as Dulbecco's Phosphate Buffered Saline solution 1X with calcium and magnesium filtered with a 0.1 µm or 0.2 µm filter. External solutions used in conventional patch clamp electrophysiology can also be used on the Barracuda system, such as HEPES-buffered balanced salt solution.

To load the external buffer boat onto the process deck:

1. Unwrap a new buffer boat, or use a clean, dry boat.
2. Push back the spring-loaded indexer at the right-rear corner of the buffer boat station nest.
3. Slide the boat into the plate nest and release the indexer.
4. Fill the boat with 25 mL to 125 mL of external buffer.

   Less than 5 mL of buffer are used for each experiment, but there must be adequate volume to evenly wet the entire bottom of the reservoir. Extra volume protects against changes in osmolality as buffer evaporates over the course of several hours.

Preparing Compound Plates

The protocol specifications in the software allow up to eight compound additions.

When preparing compound plates, you must use adequate volumes of compound, and you must have at least one plate loaded in the Compound 1 or 2 station. If you do not have a compound plate loaded in the station you specified in the Compound Addition section of the Channel Protocol Editor, the system produces an error and the experiment cannot continue.

Note: For 384-well compound plates there is a one-to-one correspondence between the compound wells and the PatchPlate wells. Any replicates must be set up in the compound plate.

To ensure proper functioning of the pipettors, the compound plate you choose must match one of the types available in the Plate Type list in the Compound Addition section of the Channel Protocol Editor:

- BD-Falcon 384W Flat Clear PPN
- Corning 384W Round Clear PPN
- Greiner 384W Flat Clear PPN
- Greiner 384W Conical Clear PPN
- Greiner 384W Small Volume Clear PS
- Greiner 384W Flat Clear PS
- Greiner 384W Conical Deep Well Clear PPN
- Nunc 384W Round Natural PPN
- Matrix 384W Reservoir V-Bottom PPN
- Axygen Low Profile Reagent Reservoir PPN

CAUTION! Fill the boat or plate with adequate volume. Inadequate volume can cause the pipettor head to aspirate an air and fluid mixture that can ruin an experiment by dispensing air into the PatchPlate wells.

Loading Compound Plates

To ensure proper functioning of the pipettes within the compound plate wells, the compound plate must match one of the types available in the Plate Type list in the Compound Addition section of the Channel Protocol Editor.

To load a compound plate:

1. Prepare your compound and fill the compound plate before loading the plate onto the process deck.

   CAUTION! Fill the boat or plate with adequate volume. Inadequate volume can cause the pipettor head to aspirate an air and fluid mixture that can ruin an experiment by dispensing air into the PatchPlate wells.

2. Push back the spring-loaded indexer at the right rear corner of the Compound 1 or Compound 2 nest.
3. Slide the compound plate into the plate nest and release the indexer. Make sure you correctly position the **A1** well in the lower-left corner of the nest.
4. If applicable, repeat the steps for a second compound addition.
5. Note the position of the compound plate on the process deck (Compound 1 or Compound 2 position).
6. Enter the plate position in the **Compound Addition** section of the Channel Protocol Editor in the **Aspirate** section **Compound Source** list (PLATE 1/PLATE 2).
7. Click the specific plate type in the **Aspirate** section **Plate Type** field.

If you are using three or more compounds in an assay, follow these additional steps:

1. In the Aspirate section of the Channel Protocol Editor, make sure you select the **Change Plate Before Aspirate** check box for your remaining compounds.
2. When the instrument pauses, remove the first two compound plates from the process deck. Load two more compounds into the Compound 1 and Compound 2 positions.
3. Adjust the **Compound Source** and **Plate Type** selections in the Channel Protocol as applicable for each of the additional compounds.

Preparing the Cell-Handling Components

The IonWorks Barracuda System uses the CellPettor subsystem for automated cell-delivery. See Using the CellPettor on page 112. Alternatively, you can choose to bypass the CellPettor and manually deliver cell suspension to the cell boat. See Delivering Cells Manually on page 113.

WARNING! Always wear protective equipment and review all applicable laboratory site regulations when handling cell solutions and cell waste. Always follow the appropriate procedures for the biosafety level of the substance you are handling.

Preparing Cell Suspension

Develop and use the cell preparation techniques optimized for the cell line and the ion channel you are investigating. The following cell dissociation and suspension method is provided for reference.

To prepare the cell suspension:

1. Pre-warm external buffer (such as DPBS) and cell dissociation agent (such as Versene, Accutase, EDTA, or Trypsin) to room temperature.
2. Remove media from the cell culture flask and rinse the cells with 10 mL of DPBS without magnesium or calcium.
3. Add 2 mL to 3 mL of cell dissociation agent to lift the cells. Limit the time the cells are exposed to the cell dissociation agent to ensure the health of the cells.
4. Observe the cells under a microscope for rounding up. Tap the flask to dislodge the cells.
5. Add approximately 10 mL of external buffer or media containing serum and triturate gently.
6. Remove 10 mL of cells to a 15 mL conical tube.
7. Centrifuge the cells for 3 to 5 minutes at 1000 rpm.
8. Decant the supernatant and, if using media, remove as much media as possible.
9. Resuspend the cell pellet in 5 mL of external buffer. Triturate the cells using a standard 200 µL hand-pipettor 5 to 10 times or more, as applicable. Different cell lines require different degrees of forcefulness and lengths of time for trituration.
10. Check the cell count. Molecular Devices recommends a cell density of 0.5×10^6 to 2×10^6 cells per mL. Use the higher range of cell densities for PPC plates. Optimize for your cell line and ion channel of interest.

    Note: Higher cell densities can result in reduced potency measurements for some compounds due to non-specific binding of compound to excess cells. Examples include hERG inhibitors.

11. Pipette the solution to a 15 mL conical tube. The minimum working volume of cell suspension is 5 mL. The maximum fill volume for the conical tube is 10 mL.

    Note: The conical tube can contain no more than 10 mL of cell suspension or it will overflow when the aspirating straw is lowered into the conical tube.

Next, load the conical tube into the cell tube holder for use with the CellPettor, or dispense manually to the cell boat.

Using the CellPettor

The automated CellPettor follows the cell-delivery procedures that you specify in the Setup Protocol.

To use the CellPettor to deliver cell suspension to the cell boat, you must perform each of the following steps:

1. Load a 5 mL aspirating pipette into the CellPettor.
2. Load a clean cell boat into the cell boat station.
3. Harvest cells from their culture flask using a cell dissociation solution, wash cells to remove cell media and cell dissociation agent, and resuspend cells to the correct final density in a 15 mL conical tube. The conical tube must contain a minimum of 5 mL of cell suspension. See Preparing Cell Suspension on page 111.
4. Load the conical tube into the cell tube holder.

Note: The conical tube can contain no more than 10 mL of cell suspension or it will overflow when the aspirating straw is lowered into the conical tube.

CAUTION! The CellPettor cell-handling subsystem requires the use of Molecular Devices approved cell boats [Standard cell boat: Molecular Devices PN 5005252]. Using labware other than the Molecular Devices approved cell boats could damage the CellPettor.

Loading the CellPettor Pipette

The CellPettor subsystem must be loaded with a clean, standard 5 mL aspirating pipette. Make sure you remove any cotton or other obstruction from the end of the pipette before you use it in the CellPettor.

CAUTION! Cotton plugs, or obstructions of any type, must be completely removed from the end of the aspirating pipette before it can be used in the CellPettor. Failing to remove obstructions could damage the equipment.

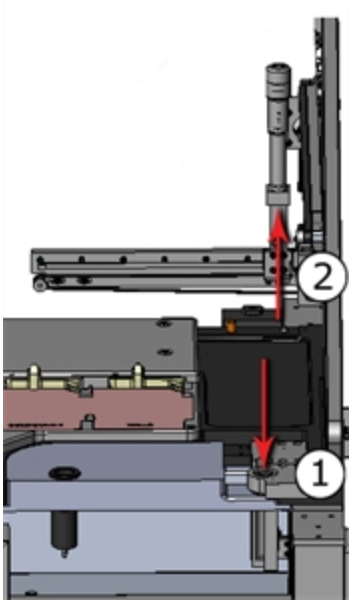
To load the aspirating pipette into the CellPettor:

1. In the shortcut bar, click  , and then click **Load CellPettor Pipette**.

   The **Preparing to load CellPettor pipette** dialog appears.
2. If needed, now is the best time to click **Quit**. Once you click **Continue**, the instrument pauses.

When the instrument pauses, the **Instrument Paused** dialog appears, the door unlocks and the pipettor robot moves into loading position.

3. If you click **Terminate**, you must run **Utilities > Calibrate CellPettor** before you can run **Utilities > Prime CellPettor**, or run an experiment.
4. Open the instrument front door. Holding the pipette with the tip pointed toward the ground, thread the pipette tip down through the tip sensor. (See arrow 1 in the diagram.)



5. Slide the end of the pipette up through the threading forks and into the CellPettor tube. Push the pipette upward with moderate pressure until you feel resistance. (See arrow 2 in the diagram.)
6. If applicable, hold the pipette mounting tube firmly and adjust the height of the pipette up or down.
7. Close the instrument front door and click **Continue** to complete the loading. Automatically, **Initialize** runs, then **Calibrate CellPettor** runs.

After loading the CellPettor pipette, continue with normal operations.

Delivering Cells Manually

If you are manually delivering cell suspension to the cell boat without using the CellPettor subsystem, make sure you click **Do not use CellPettor** in the software.

To deliver cell suspension to the cell boat manually:

1. Prepare your cells.
2. Select the **Do not use CellPettor** check box in the Setup Protocol.

3. Select an approved cell boat from the **Cell boat type** field.

> **CAUTION!** Use only Molecular Devices approved cell boats as listed in the software. Using unapproved cell boats can damage the pipettors.

4. Load a Molecular Devices approved cell boat into the cell boat station.
5. Manually pipette the cell suspension to the cell boat.

Positioning the Electrode-Plate

Despite its heavy frame, the Electrode-Plate is a delicate piece of equipment that must be handled with care at all times.

To avoid damage to the Electrode-Plate:

- Do not touch the electrode pins or the pogo pins on the underside of the Electrode-Plate.
- Keep the pogo pins dry.
- Never immerse the Electrode-Plate in liquid of any kind. The electrode pins, however, can be immersed in a bleach solution for rechloriding.
- Always lift the Electrode-Plate using the handles on the top.
- Always set the Electrode-Plate on a flat surface resting on its four stand-offs or on the Bleach Boat.

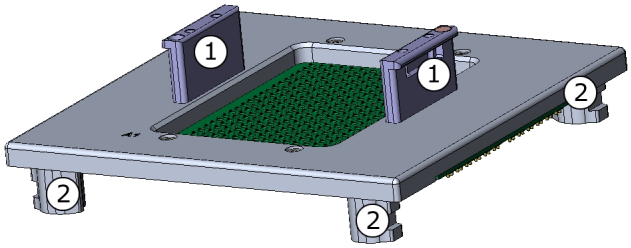


Figure 8-1: Electrode-Plate

Position	Component
1	Handles
2	Stand-offs

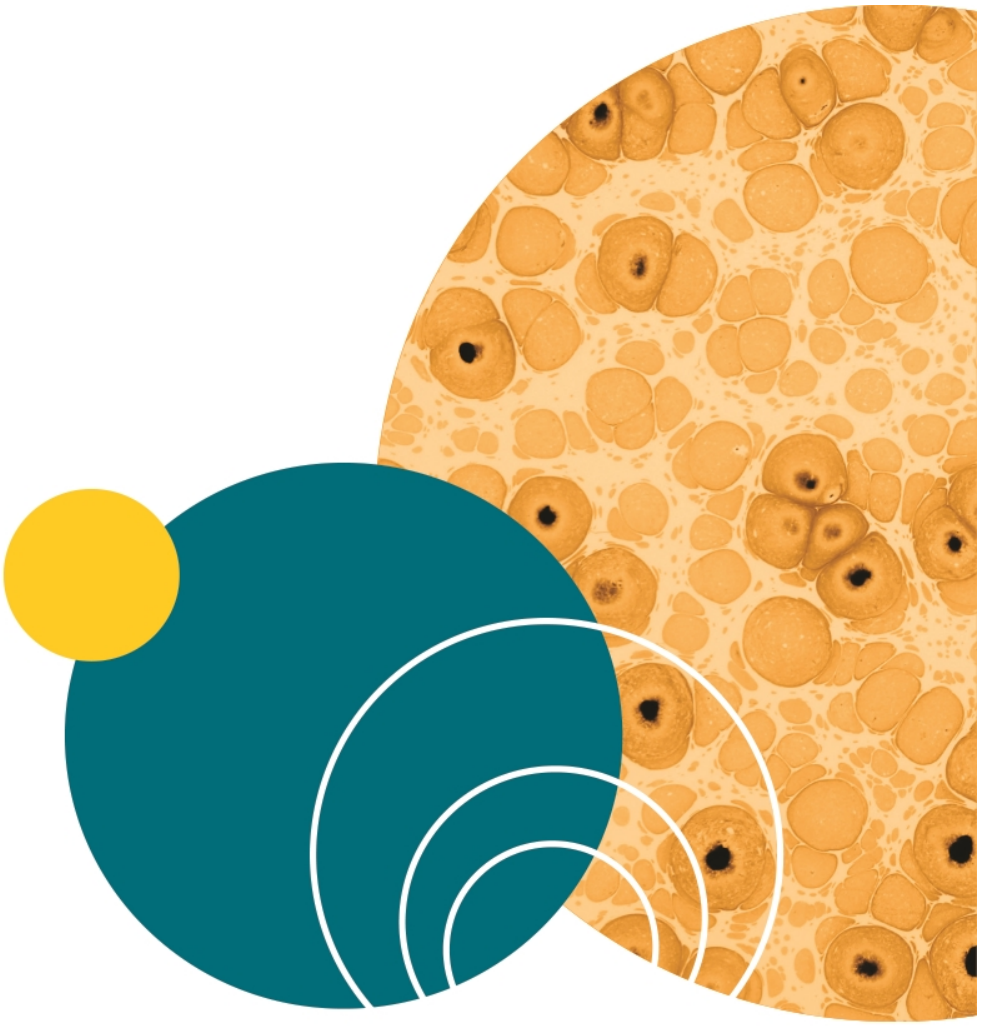
CAUTION! To avoid damage to the Electrode-Plate:

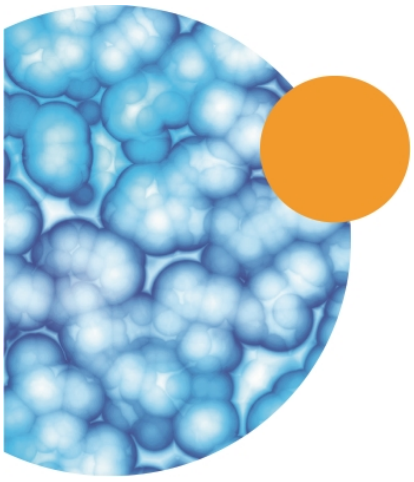
- Do not touch the electrode pins or the pogo pins on the underside of the Electrode-Plate.
- Keep the pogo pins dry.
- Never immerse the Electrode-Plate in liquid of any kind. The electrode pins, however, can be immersed in a bleach solution for rechloriding.
- Always lift the Electrode-Plate using the handles on the top.
- Always set the Electrode-Plate on a flat surface resting on its four stand-offs or on the Bleach Boat.

Placing the Electrode-Plate on the Wash Station

When you are preparing the deck for experiments, place the Electrode-Plate in its home location on the wash station. The stand-offs for the Electrode-Plate are designed to prevent incorrect placement. Locate the **A1**-designated corner and position the Electrode-Plate with the **A1**-corner in the left-front position.

During an assay, the pipettor-head moves the Electrode-Plate to the plenum base where the pogo pins connect to the amplifiers, and the electrode pins are submerged in the PatchPlate wells. During wash procedures, the pipettor-head moves the Electrode-Plate back to its resting position at the wash station.





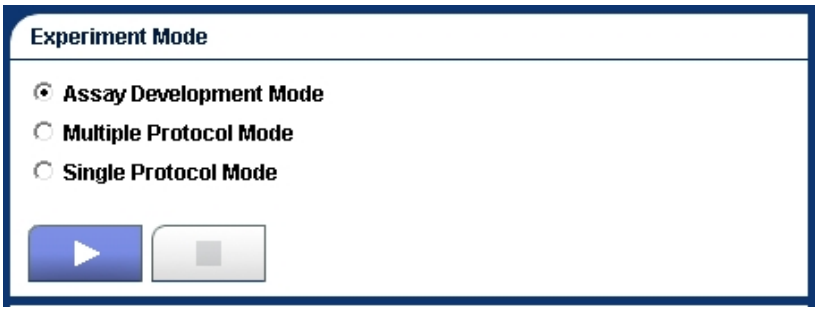
Chapter 9: Running an Experiment

9

After the instrument is prepared with fluids and labware, you can run an experiment. See Preparing the Instrument for an Experiment Run on page 101. By default the IonWorks Barracuda instrument starts in Manual mode.

To run an experiment in Manual mode:

1. Set the experiment name. For instructions, see Setting the Experiment Name on page 118.
2. Select Setup, Channel, and Cleanup Protocols. For instructions, see Selecting Protocols on page 118.
3. Select the experiment mode:



- **Assay Development Mode:** During Assay Development Mode, the selected Channel Protocol runs and pauses. At the pause, you can adjust parameters to modify the Channel Protocol. The assay continues to pause after each iteration of the Channel Protocol until you have optimized for your desired result. You can then save the optimized Channel Protocol as a new protocol, which can be run later in Multiple Protocol mode or Single Protocol mode. See Modifying a Channel Protocol in Assay Development Mode on page 47. If you are running in Assay Development Mode, follow the prompts at the pause to run through iterations of the Channel Protocol, or complete the experiment by proceeding to the Cleanup Protocol.
- **Multiple Protocol Mode**: In Multiple Protocol mode, you can select multiple Channel Protocols to run without interruption, except for specified pauses in operations to add or change compound plates or to add cells. Experiments are conducted using one Setup Protocol, any number of Channel Protocols, and one Cleanup Protocol.
- **Single Protocol Mode**: In Single Protocol Mode, the selected protocols run without interruption. One Setup Protocol, one Channel Protocol, and one Cleanup Protocol run through to completion, except for specified pauses in operations to add or change compound plates or to add cells.

4. Click the  start button. The button color varies depending on the selected mode (**Multiple Protocol Mode** or **Single Protocol Mode**).

5. Add notes about the experiment in the **Edit Experiment Notes** dialog, and then click **Continue**. To proceed without adding notes, click **Continue**.

Setting the Experiment Name

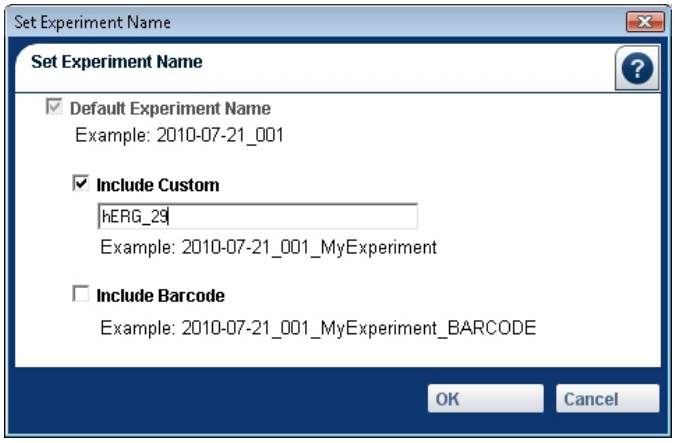
When you run an experiment, the acquired trace data is saved by default to a folder under My Documents:

C:\...\My Documents\Molecular Devices\IonWorks Barracuda\Data\2010-08-13_001

The default folder is named with the format YYYY-MM-DD_[experiment number] where 001 is the first experiment in the series. You can append more information onto the folder and file name by setting the experiment name.

To set the experiment name:

1. In the **Experiment** section of the Data Acquisition and Review workspace, click **Set**.
2. Click **Include Custom** and type any additional information into the text field that you would like to append to the date format.



3. If you are using barcoded compound plates, you can append the barcode number to the file name by clicking **Include Barcode**. The location of the compound plate barcode must be set in your Channel Protocol. When the experiment runs, the software pauses and notifies you when it is time to enter the barcode.

   Note: If **Channel Protocol > Aspirate > Barcode Position** is set to **NONE**, when the experiment runs, no prompt to enter a barcode appears, and appending a barcode number is excluded.

4. Click **OK**.

The acquired data is saved as a binary file that is not intended for editing. To export data for editing, see Exporting Data on page 143.

Selecting Protocols

In the **Protocol** section of the Data Acquisition and Review workspace, for each experiment, you must select the following from the list of saved protocols:

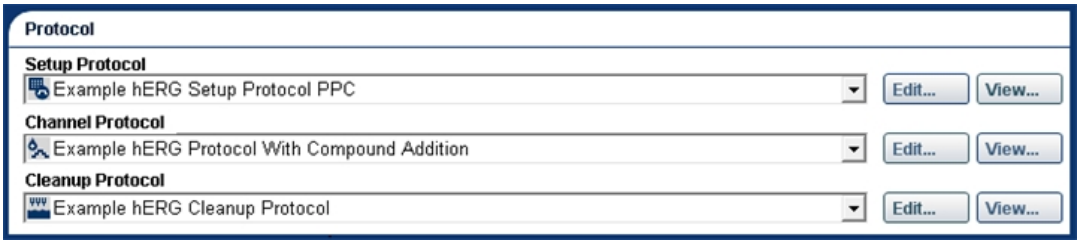
- A **Setup Protocol**
- A **Channel Protocol** (Single or Multiple)
- A **Cleanup Protocol**

Generally, the Setup and Cleanup Protocols are used repeatedly to handle common operations. The Channel Protocol you select should be specific to the type of assay you are running. You can select from a list of saved **Example** protocols provided by Molecular Devices. You can also select from the list of any edited protocols you have named and saved. See Developing New Protocols on page 45.

You can select Multiple Channel Protocols to run consecutively if you run the experiment in **Multiple Protocol Mode**. For more information, see Creating Multiple Channel Protocols on page 93.

To select protocols:

1. From the Data Acquisition and Review workspace, in the **Protocol** section, select a protocol from the **Setup Protocol** field. To see the predefined parameters for the selected protocol, click **View**.



2. In the **Experiment Mode** section, select **Assay Development Mode**, **Multiple Protocol Mode**, or **Single Protocol Mode**. Depending on your selection, either the **Single Channel Protocol** field, or **Multiple Channel Protocol** field appears in the **Protocol** section.
3. Select a protocol from the **Channel Protocol** field. To see the predefined parameters for the selected protocol, click **View**.

   Note: For instructions on setting up and saving a re-usable **Multiple Channel Protocol**, see Creating Multiple Channel Protocols on page 93.
4. Select a protocol from the **Cleanup Protocol** field. To see the predefined parameters for the selected protocol, click **View**.

Saved protocols reside by default in the My Documents folder:

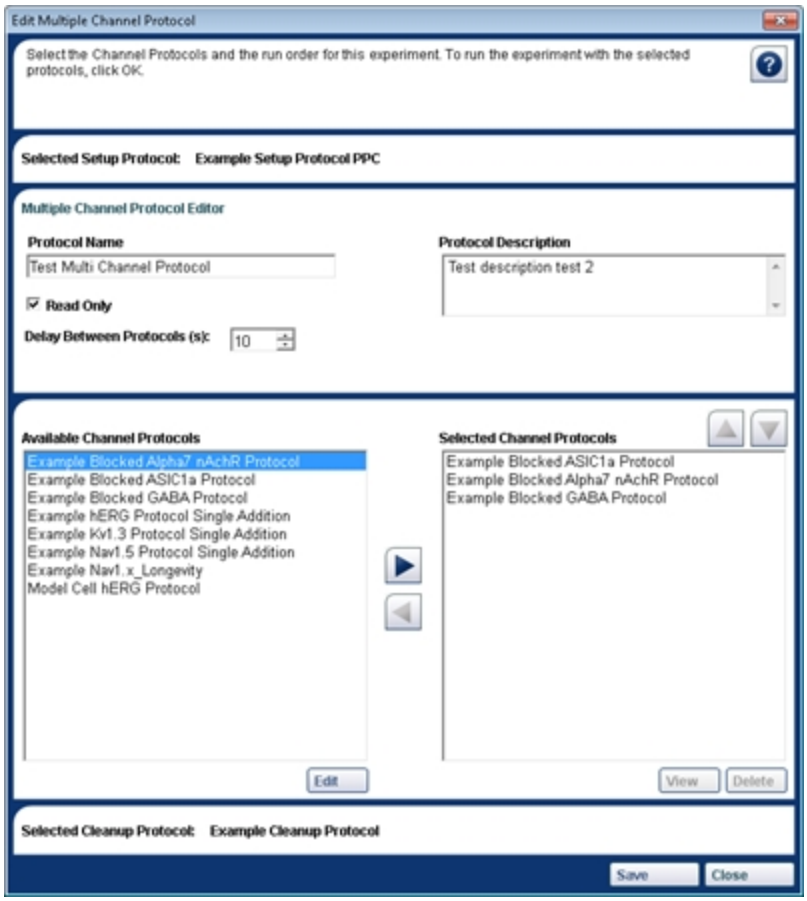
C:\Users*your user name*\Documents\Molecular Devices\IonWorks Barracuda 3.0\Protocols

Selecting Multiple Channel Protocols

When you run an experiment in **Multiple Protocol Mode**, you can select multiple channel protocols to run consecutively.

To select multiple channel protocols:

1. Start an experiment in **Multiple Protocol Mode**. For instructions, see Running an Experiment on page 117.
2. Select additional Channel Protocols to run in the experiment from the **Available Channel Protocols** list, and then click **OK**. The Channel Protocol selected in the Protocol area appears first in the **Selected Channel Protocol** list.



3. Click **Save** to finish.

Adding and Viewing Experiment Notes

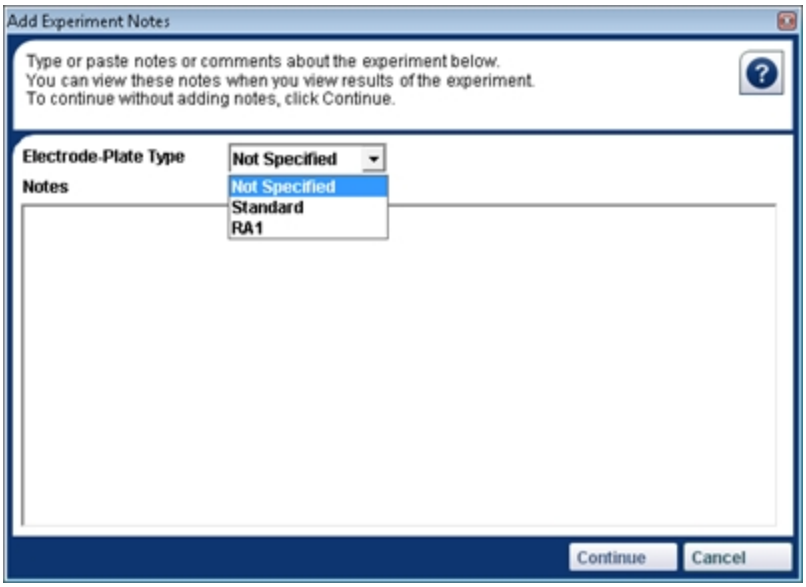
Experiment Notes are available only in the Data Acquisition and Review workspace. You can add Experiment Notes before you run an experiment, and view the added Experiment Notes after the experiment runs.

Adding Experiment Notes

Before you run the experiment, you can type or paste text notes.

To add notes to experiments:

1. Start an experiment. For instructions, see Running an Experiment on page 117
2. In the **Add Experiment Notes** dialog, specify which Electrode-Plate is used, type experiment specific comments, and then click **Continue**. Click **Cancel** to terminate the experiment. (See Electrode-Plate, see page 36.)

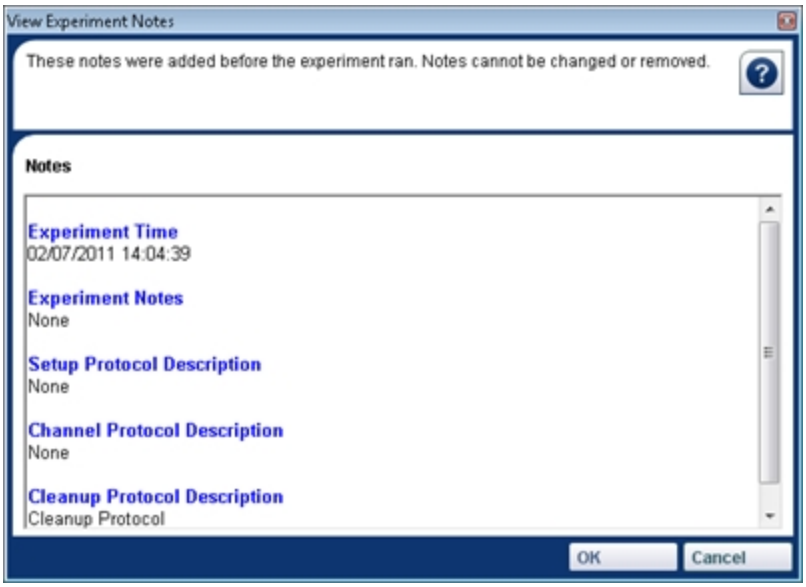


Viewing Experiment Notes

After you run the experiment, you can view the notes.

To view experiment notes:

1. In the Data Acquisition and Review workspace, open a saved experiment.
2. In the plate view, select the  tab. The **View Experiment Notes** dialog appears.



Terminating an Experiment

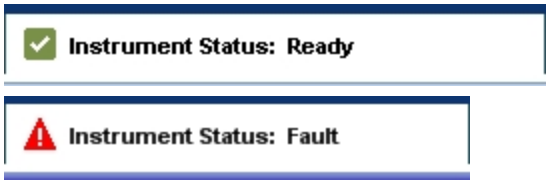
You can stop an experiment in progress in two ways:

- For non-emergency reasons, click  in the **Experiment Mode** area of the software. The instrument finishes the current action, ends the experiment, runs the cleanup protocol if needed, and returns the instrument to the **Ready** status.

  Note: The cleanup protocol needs to run only if the stop button is clicked before any fluids dispense.

- For emergency reasons, press the emergency stop button on the front panel of the instrument.
  The instrument stops abruptly, and enters a **Fault** state. To return the instrument to the **Ready** state, you must initialize the instrument. See Resetting the Instrument after an Emergency Stop on page 12 and Initializing the Instrument on page 169 for more details.

The **Instrument Status** is listed at the bottom of the main window.



Chapter 10: Viewing Acquired Data

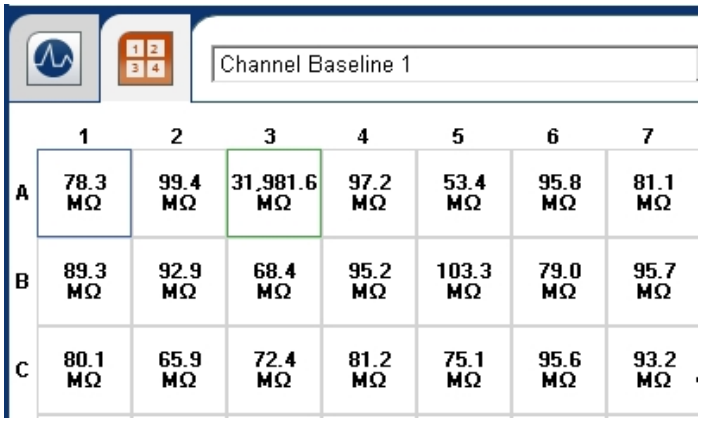
10

While an assay is running, scan results appear in the Data Acquisition and Review workspace. When the protocol completes, the results of the first scan are displayed in the plate view.

You can also view previously completed data sets by selecting a different experiment name.

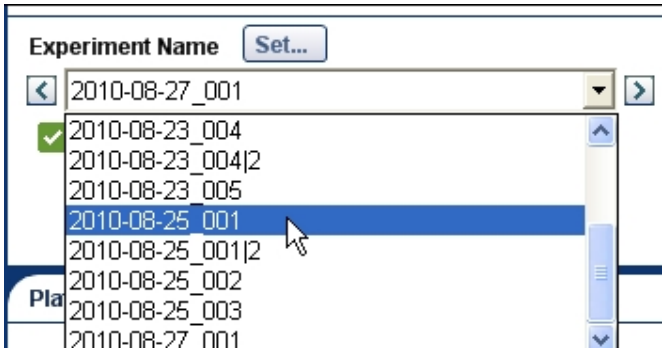
Selecting Plate View or Extracted Value View

Plate view displays the trace graphs for the selected experiment and scan. Extracted Value view displays the calculated extracted value, either for the Resistance or the Electrode Offset depending on the scan.



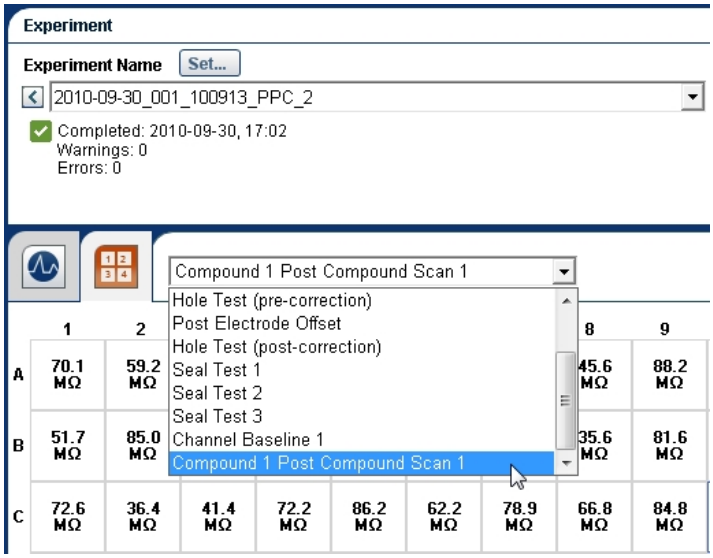
Selecting an Experiment

The most recent experiment's data is available by default in the Data Acquisition and Review workspace. To view the data from another experiment, select the experiment name from the Experiment Name list.



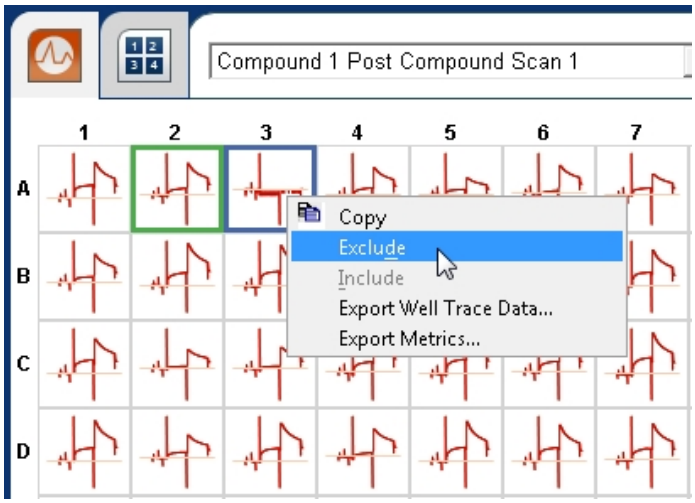
Selecting a Scan

To view results of other scans for the same experiment, click in the list of scans to select the scan you want to view. The data displayed in the Data Acquisition and Review workspace updates to the selected scan results.



Excluding Wells from Exported Data

Wells can be excluded manually from exported results.

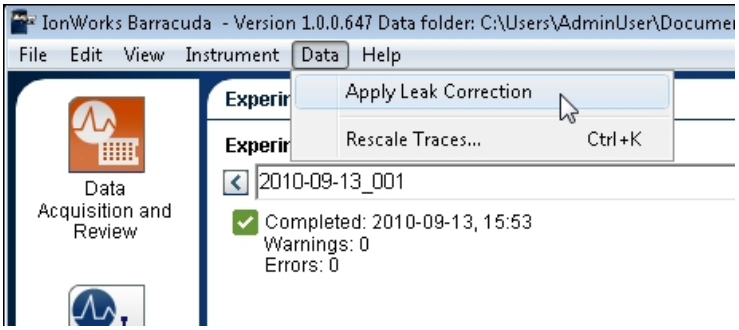


To exclude a well:

1. In the plate view, right-click the well.
2. Click **Exclude** to manually reject the well.

Applying Leak Correction

When you are reviewing data, you can turn Leak Correction on or off at any time by clicking **Data > Apply Leak Correction**.



You can also turn leak correction on or off by clicking  .

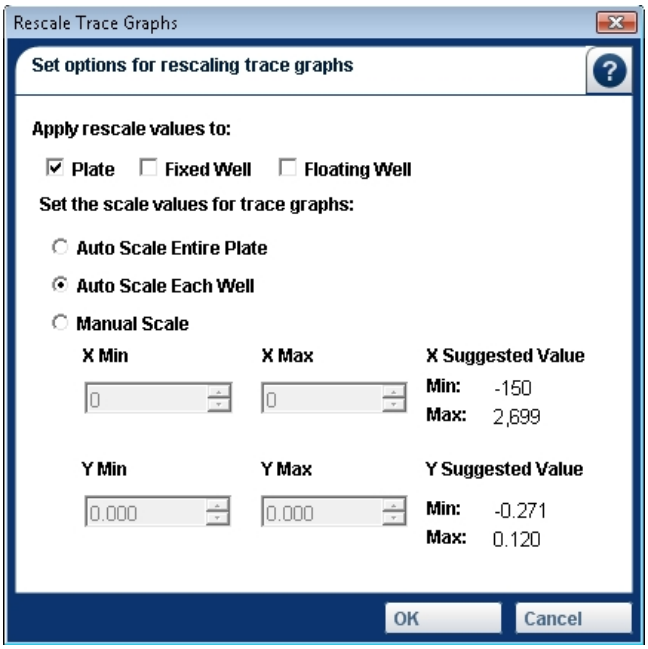
Rescaling Trace Graphs

There are many options for rescaling the trace graphs for acquired data. You can rescale each view separately, or you can apply the settings to all trace graphs. Scale settings are remembered when you switch from scan to scan or plate to plate.

To rescale the trace graphs:

1. Click **Data > Rescale Traces**, or click  .
2. Select the check boxes for Plate, Fixed Well, Floating Well as needed.

- **Plate** applies the settings you choose to the Data Acquisition and Review workspace adjusting the scale of all the well graphs in the plate.
- **Fixed Well** applies the rescale settings to the blue and green fixed well graphs on the right side of the main screen.
- **Floating Well** applies the rescale settings to the zoomed well view that opens when you double-click a well in the plate view.



3. Select the scale values to apply to the selection:

- **Manual Scale** lets you enter minimum and maximum values for the X and Y axis to scale the data according to your customized settings.
- **Auto Scale Entire Plate** sets a uniform scale value using the maximum values from all wells in the plate. When this setting is applied, it is easy to compare the relative response values between wells.
- **Auto Scale Each Well** sets a unique scale value for each well in the plate based on the maximum values for each well. When this setting is applied, the response value for each well is easier to detect and is unrelated to the scale values of the other wells.

4. Click **OK** to apply your settings.

Viewing Traces in Fixed Well View

The Fixed Well View panel uses trace graphs from wells selected in the Data Acquisition and Review workspace plate view or the Data Analysis workspace Plate Details. While viewing traces, you can change selections, zoom in, view the voltage waveform, lock the Y-axis, rescale the graphs, and switch focus between sweeps.

Selecting Traces in Fixed Well View

Trace graphs for two selected plate wells display in the Fixed Well View pane marked with a blue or a green border.

To select traces in Fixed Well View:

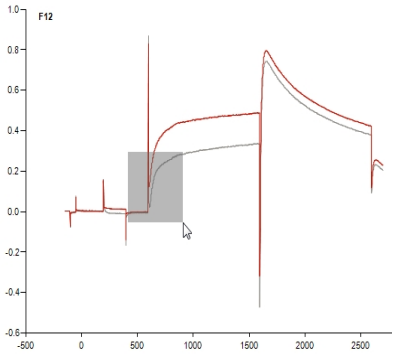
1. In the Data Acquisition and Review workspace or the Data Analysis workspace, click a plate well trace to select it. By default you are selecting with the blue border. The selected well trace appears in the Fixed Well View pane with the blue border.
2. To change the green border selection, hold down the **Alt** key and click a second plate well trace.

Zooming in on a Trace

Zoom in on any portion of a trace in Fixed Well View to show greater detail. Zoom works on both trace selections at the same time. To return to normal zoom (100%), right-click anywhere in the zoomed graph and click **Zoom Out**.

To zoom in on a trace:

1. On a selected Fixed Well View graph, click and drag the cursor to select an area you want to zoom. A gray highlight marks the selection in progress.



2. Release the mouse button. The trace graph zooms in on the selected area. Scrollbars appear along the Y–axis and the X–axis. If you are viewing scans with a compound addition, a green vertical line indicates the point in time when the compound was introduced.

Display Selected Sweep in the Plate View

In the Data Acquisition and Review workspace, when the Fixed Well View is displaying more than one sweep, you can display the selected Fixed Well View sweep in the plate view.

To display the selected sweep in the plate view:

- When the sweep numbers are different in the Fixed Well View and plate view, click  . The plate view sweep changes to match the Fixed Well View sweep.

Note: If the button is inactive, go to **Edit > Preferences** and clear the **Auto Refresh on Sweep Selection** check box.

Displaying the Voltage Waveform

The Fixed Well View can display the voltage waveform in a third pane below the selected blue and green graphs.

To display the voltage waveform:

- Click  to display or hide the voltage waveform.

Locking the Y–Axis between the Two Graphs

In the Data Acquisition and Review workspace, you can synchronize the Y–axes between the blue and green graphs in the Fixed Well view by locking the Y–axis.

- Click  to lock or unlock the Y–axis.

 **Note:** The Y–axis can be locked only in the Data Acquisition and Review workspace.

Switching Focus Between Sweeps

When the Fixed Well View is displaying more than one sweep, the blue sweep is the sweep with focus, or the sweep that is "on top." You can navigate between the sweeps by using the keyboard arrow keys to shift the focus to another sweep. Click the up-arrow or the down-arrow key on the keyboard to rotate the sweep that is currently "on top" of the stack of traces.

You can also double-click a sweep to select it for focus.

Rescale Trace Graph

Scale settings are remembered when you switch from scan to scan or plate to plate.

To rescale the trace graphs:

1. Click  .
2. Select the check boxes Fixed Well.
3. Select the scale values to apply.
4. Click **OK** to apply your settings.

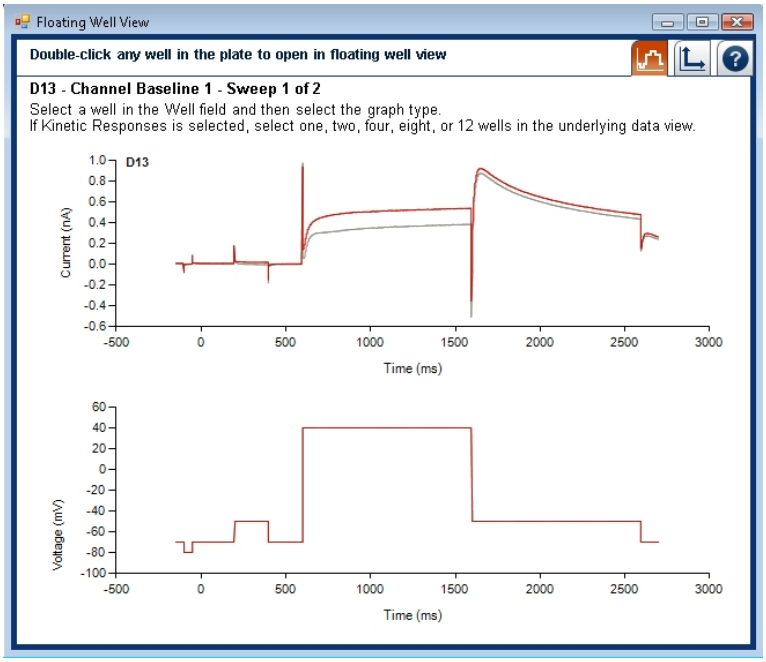
Viewing Traces in Floating Well View

The Floating Well view zooms trace graphs from a well selected from the Data Acquisition and Review workspace. Any portion of the graph in Floating Well view can be zoomed to show greater detail. To return to normal zoom (100%), right-click anywhere in the zoomed graph and click **Zoom Out**. For compound-triggered acquisitions, the green vertical line indicates the time at compound addition.

Displaying and Zooming a Well in Floating Well View

To display a well in Floating Well view:

1. Double-click any well with the left mouse button to display the selected well in Floating Well view.
2. To zoom a selection, position the cursor in the top left corner of the area you want to zoom.



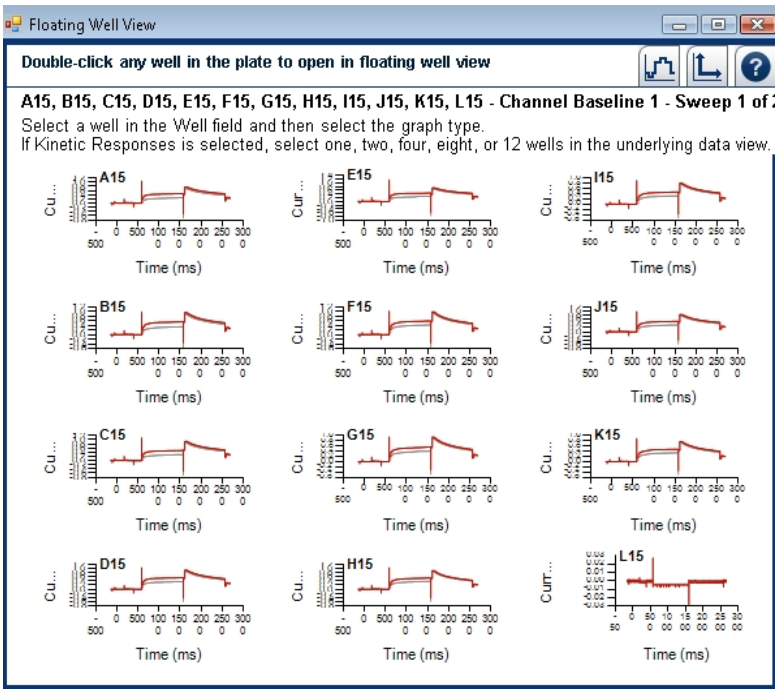
3. Click and drag the cursor down and to the right to select an area.
4. Release the mouse button. The Floating Well view is zoomed to display the selected rectangular area.

Selecting Multiple Wells in Floating Well View

You can select multiple wells to view simultaneously in Floating Well view.

To select multiple wells to view in Floating Well view:

1. While pressing the Ctrl key, click two, four, eight, or twelve wells to view.
2. Double-click the last well to open the selected wells in Floating Well View.



3. Maximize the window to enlarge the graphs.

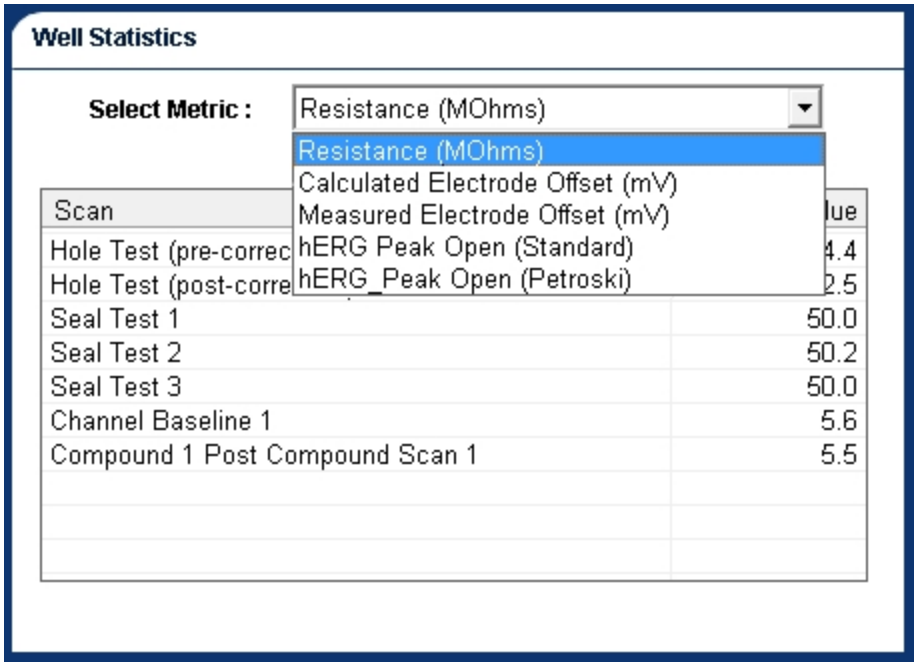
Switching Focus Between Sweeps

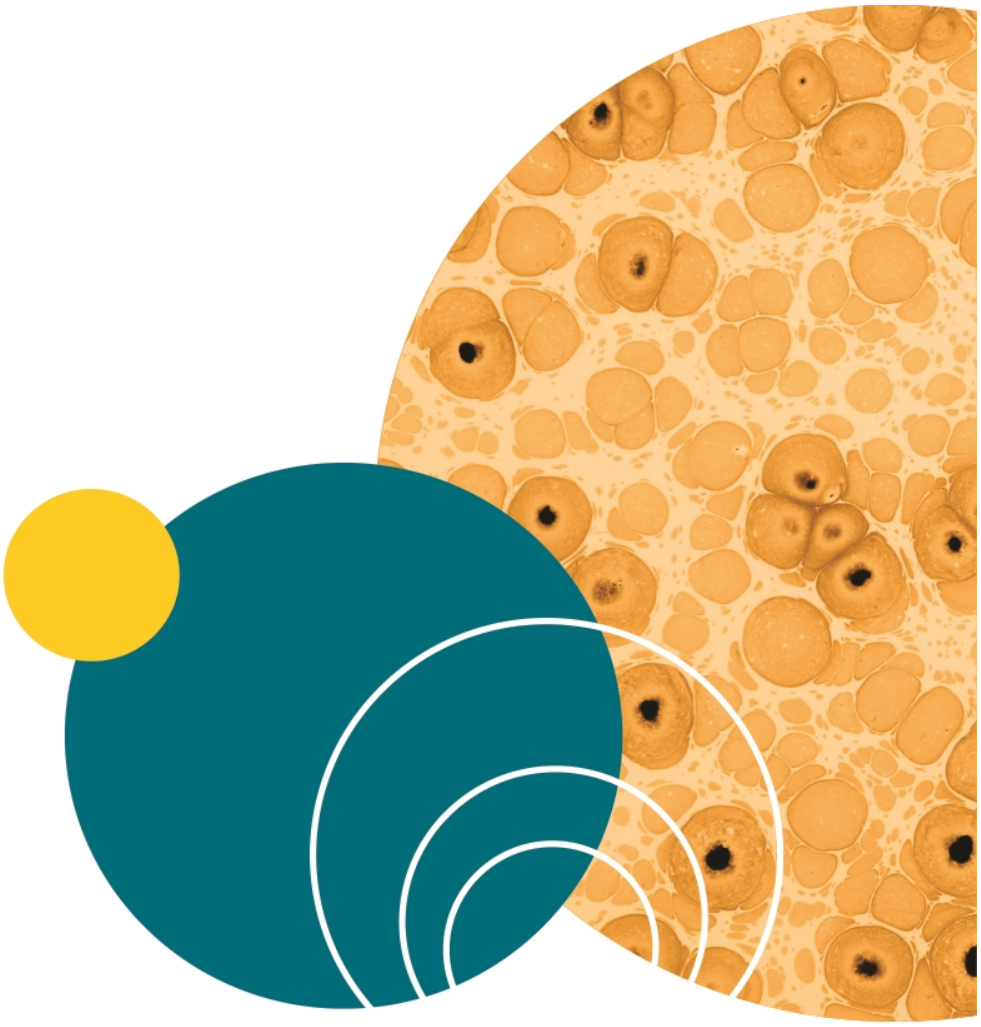
When the Floating Well view is displaying more than one sweep, the blue sweep is the sweep with focus, or the sweep that is "on top." You can navigate between the sweeps by using the keyboard arrow keys to shift the focus to another sweep. Click the up-arrow or the down-arrow key on the keyboard to rotate the sweep that is currently "on top" of the stack of traces to the red color.

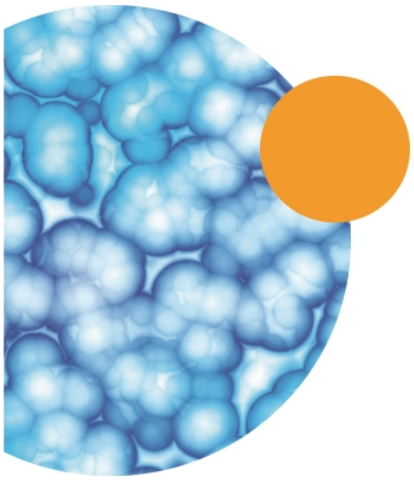
You can also double-click a sweep to select it for focus.

Viewing Well Statistics

Well Statistics appear only in the Data Analysis workspace. You can view well statistics for each metric by selecting the metric name in the **Select Metric** menu. If the entry in the Value column is **NA**, the statistic is not applicable to the scan.







Chapter 11: Analyzing Data

The **Data Analysis** workspace provides views that summarize responses and statistics based on the experiment type. You can perform basic analysis of the acquired data using metrics, filters, and summaries, and then export your results for further analysis. IonWorks Barracuda® System Software offers several data analysis methods:

- Using metrics, data can be reduced to a single value.
- Using filters, data that is not useful can be eliminated from results.
- Using summaries, hit criteria can be refined to narrow remaining results.

The first step when analyzing data is to select the completed experiment name from the **Experiment Name** field to display the data in the Plate Details area.

Using Metrics in Data Analysis

IonWorks Barracuda® System Software provides an editor for setting up metrics for analysis of acquired data. When metric settings are created, they are named and then saved with the Channel Protocol. In general, one value is output per well and per sweep for the metric.

To edit a metric while viewing data:

1. Click  to open the metric editor. Make changes as applicable.
2. **Save** the metric settings. If you do not want to overwrite the previous settings, enter a new name before saving the metric.

To create and save a metric while developing a Channel Protocol, follow the instructions for Creating a New Metric on page 86.

Metric data can be automatically exported to your preferred file location upon completion of the Channel Protocol. To configure a metric for automatic export, see Automatically Exporting Metrics Data on page 88.

You can also import saved metric settings from other Channel Protocols for reuse. To use saved metric settings in another protocol, follow the instructions for Importing a Metric on page 87.

Adjusting Native Filters

In the Data Analysis workspace, you can adjust the parameters for the seal test to increase or decrease the "No Seal" definition for your selected plate.

PatchPlate Holes: The hole test filter measures for the resistance between the Electrode-Plate electrodes and the plenum ground electrodes for each well. Resistance that measures less than 1 MΩ or greater than 10 MΩ is reported as "blocked." Resistance that is too high might mean there are air bubbles in the well. The hole test thresholds are pre-defined for PatchPlate quality control purposes and cannot be changed.

PatchPlate Seals: The seal test filter measures that the cells are properly seated over the holes in the PatchPlate wells. When data files are acquired, the definition for "No Seal" is defined as resistance that is less than 50 MΩ for SH or 20 MΩ for PPC, or greater than 1500 MΩ for either SH or PPC. You can change these settings for a given data file (ranging from 1 to 100,000 MΩ) to increase or decrease the available data from the plate.

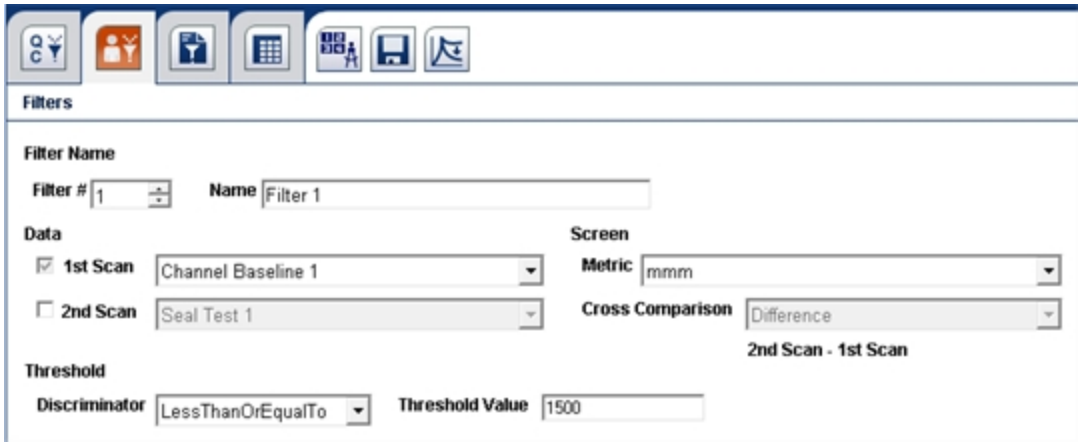
To change the pre-defined values used for the seal test:

1. Click  , and then type new values in the **less than** and the **greater than** fields.
2. Click **Save**.

Defining Filters to Analyze Data

Filters are used to reduce the number of wells included in exported data for the selected experiment. Filters are generally applied after metrics to exclude bad data and reduce the number of exported wells.

Click  in the Data Analysis workspace.



To define a new filter for the protocol:

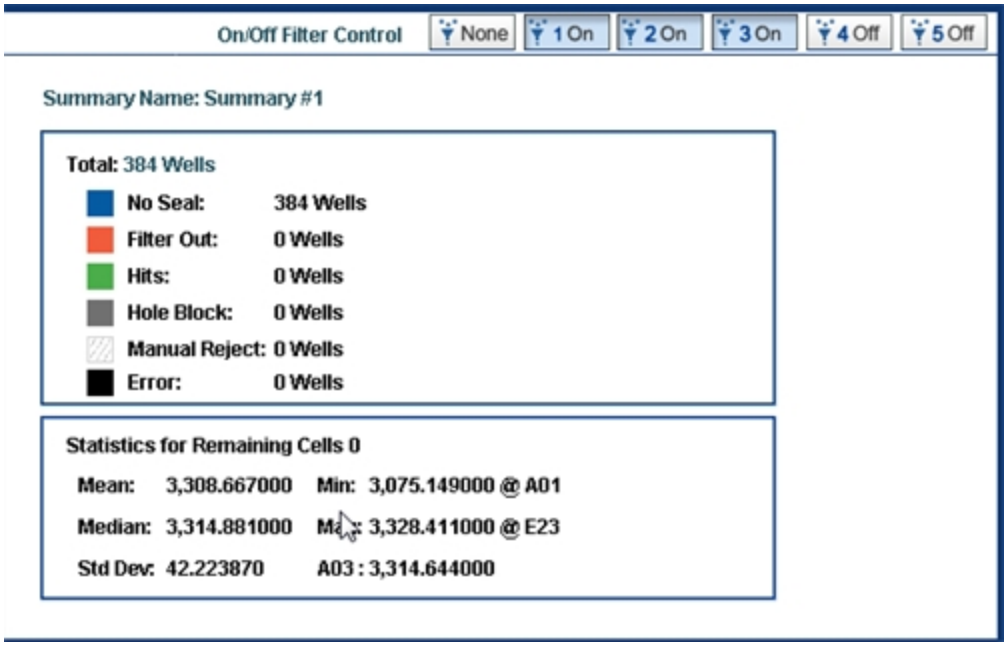
1. If you have already saved one or more filters for this protocol, click the **Filter #** field buttons to increment to a new filter number.
2. Type a description of the filter in the **Name** field.
3. Select parameters to apply to the plate data.

- Select one scan and one metric for a simple filter.
- Select two scans and one metric for a comparative filter. Select a method of comparison for the two scans. Check that the formula displayed agrees with your intended comparison method.

4. Select a condition from the **Discriminator** field.

5. Type a numeric **Threshold Value** for the results.
6. Click **Save**.

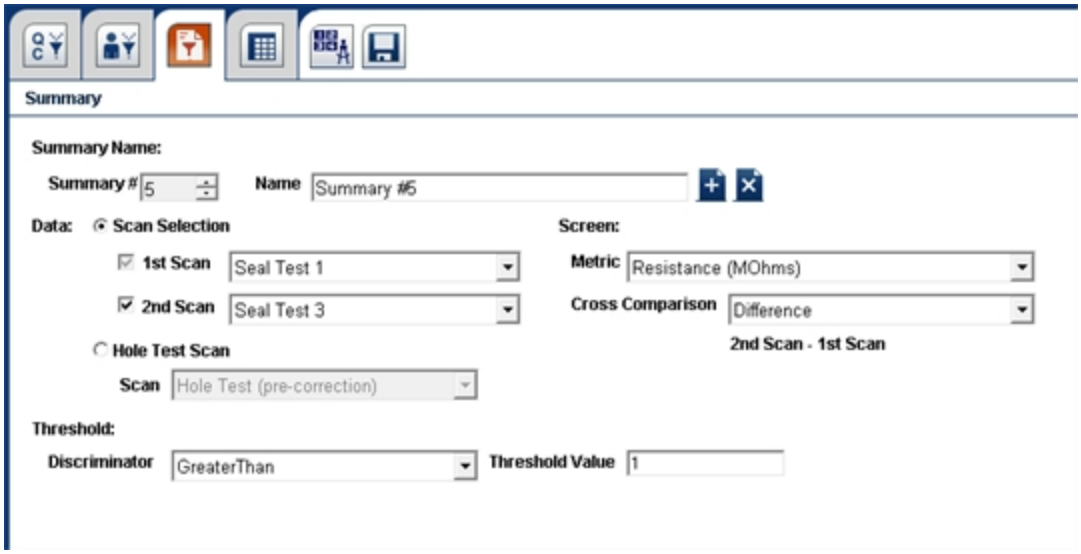
To apply the filter to the plate data, in the On/Off Filter Control area, click the button with the appropriate filter number to toggle the filter on or off.



Defining Summaries to Analyze Data

Summaries are used to refine the selection of hits included in exported data for the selected experiment. Summaries are generally applied after metrics and filters to further narrow the criteria for exported data.

Click  in the Data Analysis workspace.



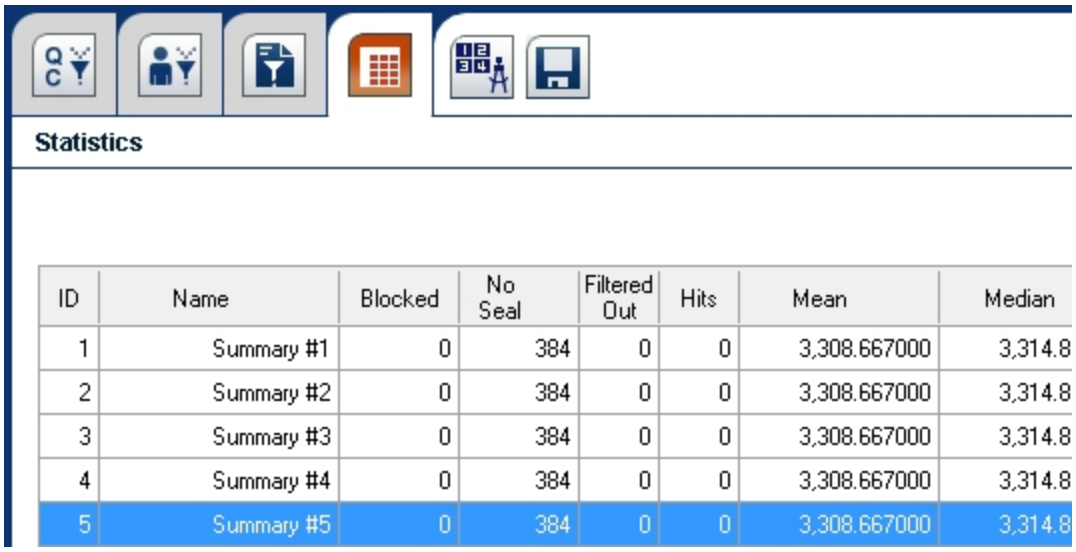
To define a new summary for the experiment data:

1. If you have already saved one or more summaries for this protocol, click the arrow buttons to increment to a new summary number.
2. Type a description of the summary in the **Name** field.
3. Select parameters to apply to the plate data.

- Select one scan and one metric for a simple summary.
- Select two scans and one metric for a comparative summary. Select a method of comparison for the two scans. Check that the formula displayed agrees with your intended comparison method.

4. Select a condition from the **Discriminator** field.
5. Type a numeric **Threshold Value** for the results.
6. Click **Save**.

To see statistics for all saved summaries, click  .



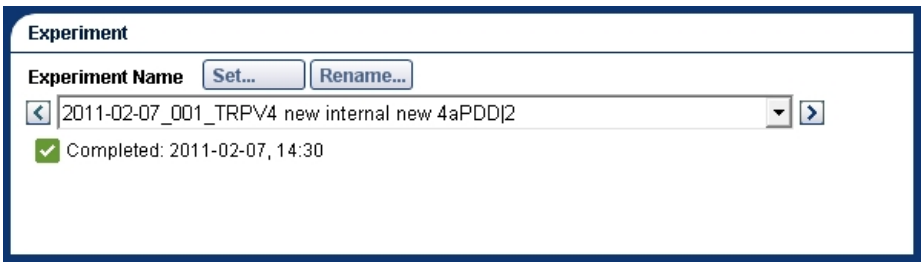
ID	Name	Blocked	No Seal	Filtered Out	Hits	Mean	Median
1	Summary #1	0	384	0	0	3,308.667000	3,314.8
2	Summary #2	0	384	0	0	3,308.667000	3,314.8
3	Summary #3	0	384	0	0	3,308.667000	3,314.8
4	Summary #4	0	384	0	0	3,308.667000	3,314.8
5	Summary #5	0	384	0	0	3,308.667000	3,314.8

Viewing Associated Protocols

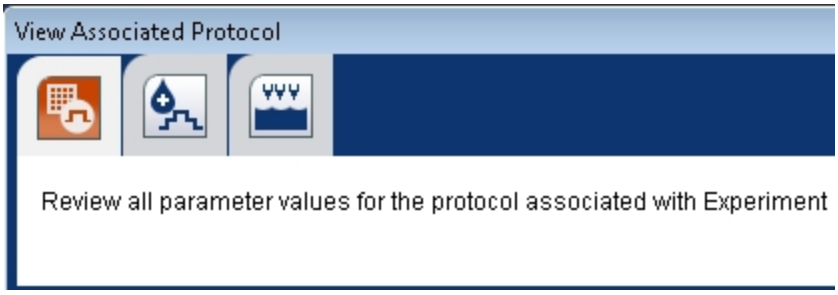
While an experiment is running, you can view acquired data for each scan as it progresses. If there is no experiment in progress, you can review the acquired data for other experiments in the Data Acquisition and Review workspace by first selecting the experiment name.

To view the protocol parameters for a plate with acquired data:

1. Select the experiment name from the **Experiment Name** field. The data for the selected data set loads in the plate view.



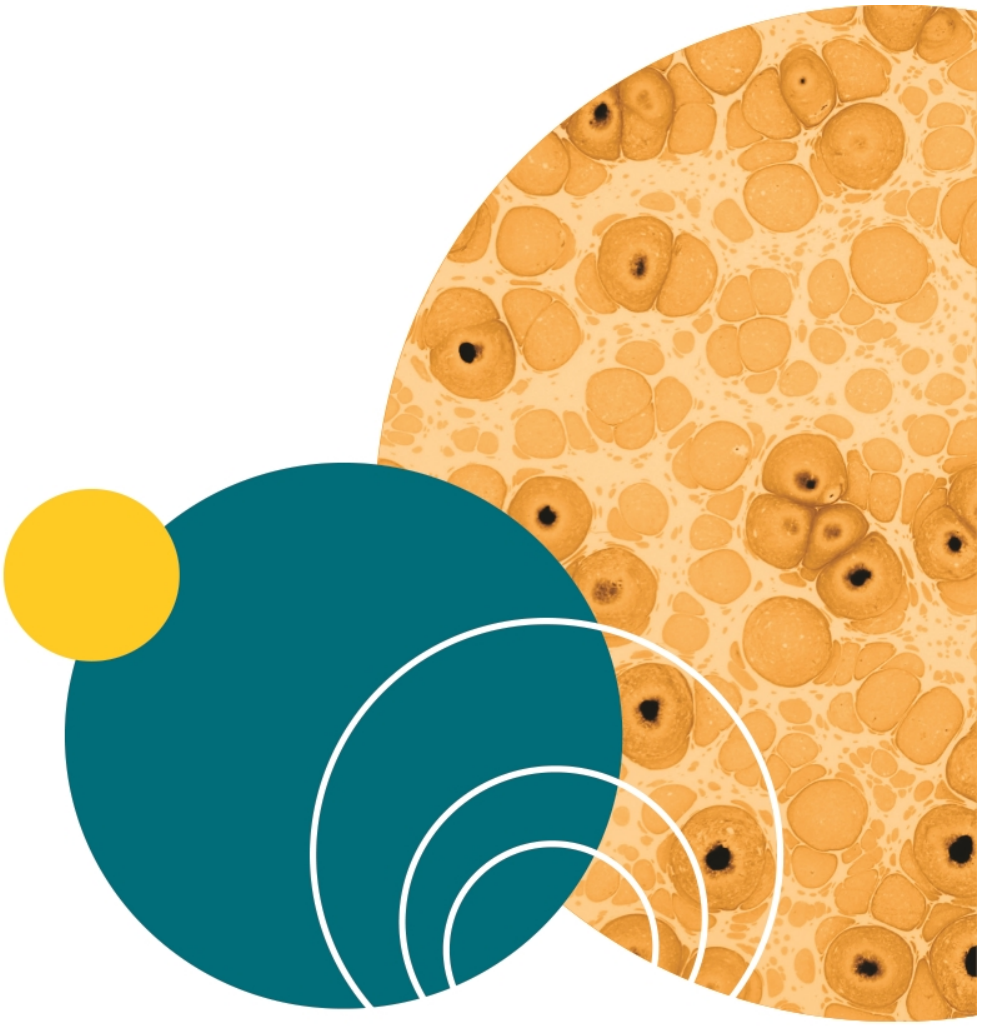
2. Click the **Associated Protocols** shortcut from the buttons on the left.
3. After the summary window appears, select the tab for the Setup, Channel, or Cleanup Protocol to display the summary details.

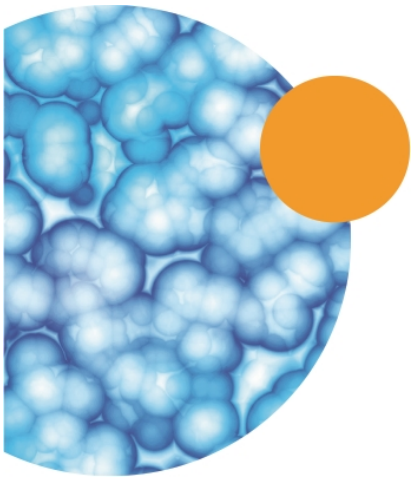


To print the summary, click  and follow the prompts.

To copy the summary to the clipboard:

1. Click  . The summary data is copied to the clipboard.
2. Paste the data into your preferred application.





Chapter 12: Managing File Locations

The IonWorks Barracuda System produces many data files of different types including:

- Raw plate data acquired during experiments
- Export data after metrics or filters are applied
- Export trace (curve) data collected for each well
- Saved Setup, Channel, and Cleanup Protocols
- Saved metric settings

Default Folder Locations

To manage this data, you can use the default folders created with the software installation, or you can set your own. If you change the default locations, the software retains the new location and subsequently saves your data there.

- The root directory is:
  C:\Users\<your user name>\Documents\Molecular Devices\IonWorks Barracuda\...

By default, files are saved in the applicable Documents location for your operating system in one of three folders:

- **\Protocols**:
  When you initially create a protocol, before it is associated with an experiment, the file is saved in the Protocols folder as
  ***protocol name.protocol type*.ibp**.
- **\Data:**
  The data folder contains plate data acquired during assays, as well as protocols after they are associated with an experiment. Whenever an experiment is run, a new folder is created with the experiment name to hold all of the various experiment data files. If you ran the protocol named Test-Protocol99 on October 31, 2012, and named the experiment My-Experiment99, the acquired data would be saved in
  ...\Data\2013-01-31_001-MyExperiment99
- **\Export**:
  The export folder holds both manually and automatically exported metric results, and exported trace data. Metric result files and trace data files can be custom-named, or can default to the experiment name.

Note: In the software menu, click **File > Set Default Folders** to view the last five saved file paths for \Protocols and \Data.

For more information about naming experiments, see Setting the Experiment Name on page 118.

Organizing Data

Every type of data produced by the IonWorks Barracuda System can be saved in a unique location by individual users. Because there are many related data types, you might want to establish and follow conventions for creating folders and saving files so that data, export files, saved metric settings, and protocols are easy to locate later.

Changing the Default Protocols Folder

By default, the IonWorks Barracuda® System Software stores saved protocols in these locations:

C:\Users\<your user name>\Documents\Molecular Devices\IonWorks Barracuda\Data

You can create folders to separate the data from different assay types, different dates, or different users.

To create a new folder for protocols:

1. Click **File > Set Default Folders**.
2. In the **Default Folders** dialog, click the **Browse** button next to the Protocols folder and then navigate to the desired parent folder.
3. Click **Make New Folder**. A folder is created named New Folder.
4. Right-click the new folder, click **Rename**.
5. Type a new folder name, and then press **Enter**.
6. Make sure the new folder is selected and click **OK**.
7. When you return to the Default Folders dialog, click **OK** again.

Changing the Default Data Folder

By default, the IonWorks Barracuda® System Software stores acquired plate data in these locations:

C:\Users\<your user name>\Documents\Molecular Devices\IonWorks Barracuda\Data

You can create folders to separate the data from different assay types, different dates, or different users.

To create a new folder for data files:

1. Click **File > Set Default Folders**.
2. In the **Default Folders** dialog, click **Browse** and navigate to the desired parent folder.
3. Click **Make New Folder**. A folder is created named New Folder.
4. Right-click the new folder, and then click **Rename**.
5. Type a new folder name, and then press **Enter**.
6. Make sure the new folder is selected and click **OK**.
7. When you return to the **Default Folders** dialog, click **OK** again.

To set the location for exported metrics, see Exporting Metrics Data on page 143.

To set the location for exported trace data, see Exporting Trace Data on page 146.

Naming Conventions for Files

Within each of the default (or custom location) folders, files are saved with naming conventions designed to help you identify them later. The default file location for saved files is:

C:\Users*your user name*\Documents\Molecular Devices\IonWorks Barracuda\[Data, Protocols, or Export Folder]

Note: If the default folder locations for DATA, PROTOCOLS, or EXPORT are changed, then the files are saved to the new folder locations.

For each experiment, the generic name for files is the date (YYYY-MM-DD) followed by the series number for the experiment (_001, _002, _003...). If you give the experiment a custom name, then the custom name is added to the generic experiment name:

YYYY-MM-DD-001-custom-experiment-name...

You can also add a barcode number to the experiment name. For more information about naming experiments, see Setting the Experiment Name on page 118.

The examples in the following table include a custom experiment name.

Data Folder

Whenever an experiment is started, a folder is created within the **\DATA** folder that is named by the experiment name. All series data and assay development data is subsequently saved in the experiment-name folder.

The naming conventions for files in the **\DATA\EXPERIMENT-NAME** folder include:

Type of Data	File Extension	Naming Convention for Files
Raw plate data	.ibe	**YYYY-MM-DD-001-custom-experiment-name.protocol-type.ibe** where 001 is the series number for the experiment. Changes to 002 when run again.
Raw plate data for Channel Protocols	.ibe	**YYYY-MM-DD_001-custom-experiment-name.Channel.1.ibe** where .1 is the saved data from the first iteration run during assay development mode. Changes to .2 when the assay is run again.
Protocols	.ibp	**YYYY-MM-DD_001-custom-experiment-name.protocol-type.ibp**
Metrics settings	.iba	**YYYY-MM-DD_001_custom-experiment-name.metric-name.iba**

Type of Data	File Extension	Naming Convention for Files
Plate information	.xml	**YYYY-MM-DD_001_custom-experiment-name.PlateInfo.xml** Includes the original protocol name and other experiment details.

Protocols Folder

When a protocol is saved and named, before it is associated with an experiment, it is saved in the **\PROTOCOLS** folder along with the metric settings created with the Channel Protocol.

Files found in the **\PROTOCOLS** folder include:

Type of Data	File Extension	Naming Convention for Files
Protocols	.ibp	**protocol-name.protocol-type.ibp**
Metrics settings	.iba	**protocol-name.iba**

Export Folder

Exported metric results are saved by default in the **\EXPORT** folder. Naming conventions for the exported data files include:

Type of Data	File Extension	Naming Convention for Files
Automatically exported metric data	.csv or .txt	**YYYY-MM-DD_001-experiment-name.protocol-type.ibp**
Metric export data	.csv or .txt	**experiment-name.csv** or **custom-name.csv**
Trace data for each well	.csv or.txt	**\well#-folder\YYYY-MM-DD_001-experiment-name.scan-type.scan#.csv**
Plate information	.xml	**YYYY-MM-DD_001_experiment-name.PlateInfo.xml** Includes the original protocol name and other experiment information

Chapter 13: Exporting Data

13

Several types of data can be exported from the IonWorks Barracuda System in various formats. Exported metric and trace data can then be imported into other software for further analysis.

Types of data include:

- **Exported Metric Data:** Data that has one or more metrics applied to reduce it to a single numeric value.
- **Automatically Exported Metric Data:** Data that has one or more metrics applied and is output at the time the Channel Protocol completes.
- **Trace Data:** Curve points for sampled data from individual wells acquired during scans.

Formats include:

- CSV: Comma-delimited data
- TXT: Tab-delimited data

Each set of data is saved in a folder designated at the time of export. The default folder location for exported files is:

C:\...\Documents\Molecular Devices\IonWorks Barracuda\Export

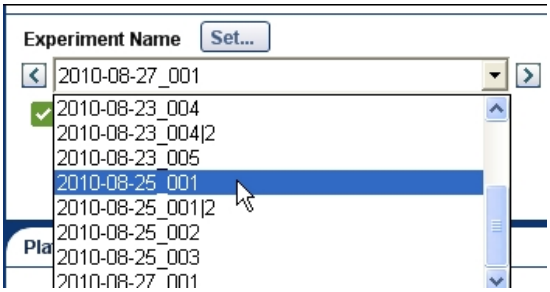
Exporting Metrics Data

After a protocol completes, data can be manually exported from the results at any time. The exported data can first have metrics, filters, and summaries applied to produce the most useful output.

Metrics can also be defined and set up for automatic export as part of the Channel Protocol development. For information on automatically exporting data when a protocol completes, see Automatically Exporting Metrics Data on page 88.

To manually export data:

1. Select the experiment name with the data you want to export from the Experiment list.



2. Click **Metrics Export** from the list of shortcuts on the left side of screen to open the Export Metric dialog.

3. Select the data set to include in the metric export: Channel Protocol scans only or Channel Protocol scans plus hole test and seal test results.
4. Select the **Results Format**:

- **Plate** format inserts results in a spreadsheet with a separate data set for each scan in a format that duplicates the well layout: rows A-P, and columns 1-24.
- **Column Sorted by Row** format lays out the results for each well in a descending order by row, A1, A2, A3... descending to P24, with scan results arranged in the columns from left to right across the spreadsheet.
- **Columns Sorted by Column** format lays out the results for each well in a descending order by column, A1, B1, C1... descending to P24, with scan results arranged in columns from left to right across the spreadsheet.

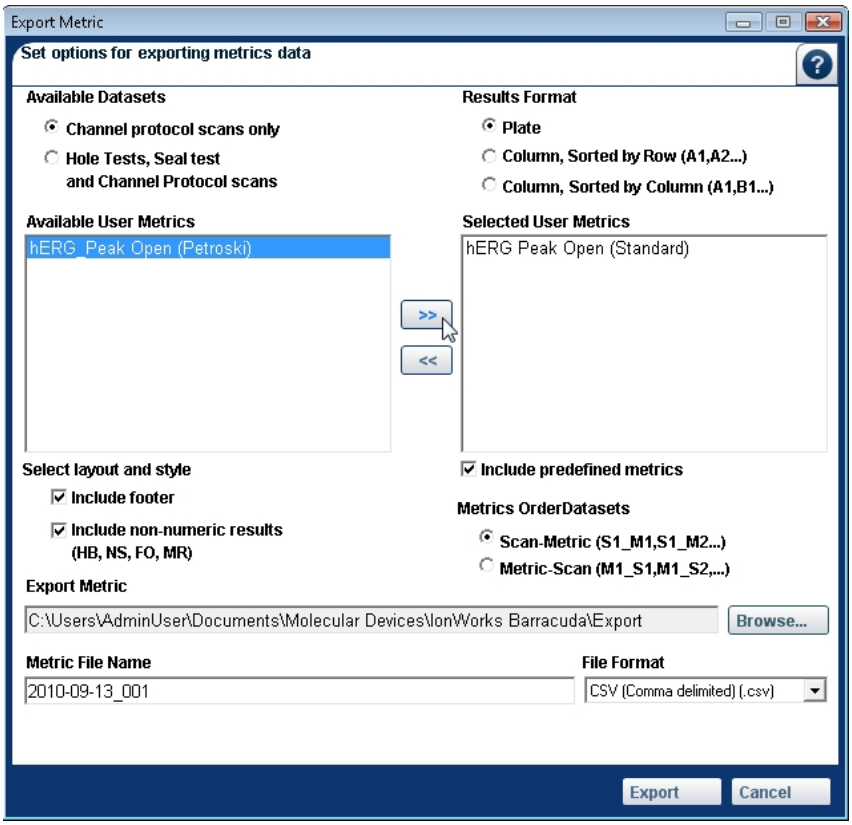
5. Select the metric you named and defined from the list of **Available User Metrics**.
6. Click >> to move the selected metric to the **Selected User Metrics** field.
7. Check the boxes to include or exclude footer data. Footer data includes:
   A key to the column names for included scans, sweeps, and metrics.
   A key to non-numeric results: HB-hole blocked, NS-no seal, FO-filtered out, MR-manually rejected.
   Definitions for metric setting values and conditions.
   Protocol information including:

- Setup Data File Name
- Channel Data File Name
- Date/Time (local)
- Date/Time UTC
- Setup Protocol Name
- Channel Protocol Name

8. Check the box to **Include predefined metrics** including offset, hole test, and seal test values.
9. Select the **Metrics Order Dataset** by Scan-then-Metric values (S1_M1, S1_M2, S1_M3...) or Metric-then-Scan values (M1_S1, M2_S1, M3_S1...).
10. If needed, browse to select the folder for your exported metrics data in the **Export Metric** field. The default path setting is **C:\Users\AdminUser\Documents\Molecular Devices\IonWorks Barracuda\Export**.

Note: If you have an invalid **Export Metric** path entered, such as to a USB flash drive that has been removed, you will be prompted to change the **Export Metric** path entry to an existing directory.

11. Type a name for the exported metric data file in the **Metric File Name** field. The exported metric file is saved with the name you type. If you do not type a name, then the exported metric file is named with the experiment name.
12. Select a file format from the **File Format** field: CSV for comma-delimited data, or TXT for tab-delimited data.



13. Click **Export**. The data exports immediately to your selected folder.

> **Note:** The **Export** button is not available when you are configuring metric export options while editing a protocol.

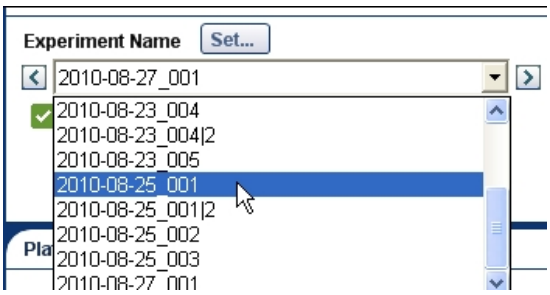
14. To view the exported data, click **File > Open Exported Data Folder** and select from the list of exported data files in the folder.

Exporting Trace Data

Trace data (curve data) can be exported for individual scans and individual wells. When traces are exported, coordinates for each sample point in the scan are included in each file. This can quickly lead to enormous amounts of very detailed data, so make sure you are selecting your trace data carefully before exporting.

To manually export trace data:

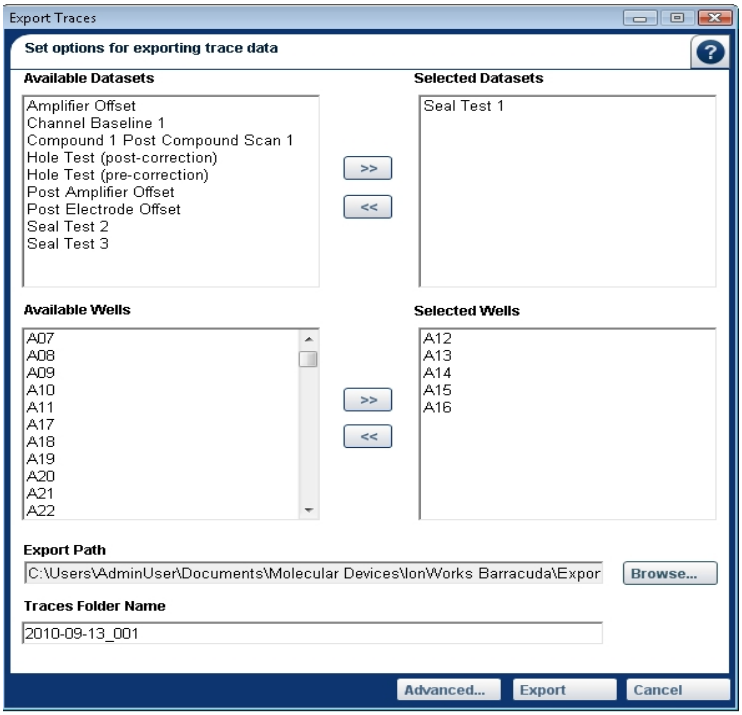
1. Select the experiment name with the data you want to export from the Experiment list.



2. In the File menu, click **Export Traces**. The Export Traces dialog appears.
3. Select the data set (scan) to include in the traces export. To select more than one, hold down the **Ctrl** key and click additional scans.
4. Click >> to move the selected data set to the **Selected Datasets** field.
5. Select the **Available Wells** to include in the traces export. To select more than one well, hold down the **Ctrl** key and click additional wells.
6. Click >> to move the selected wells to the **Selected Wells** field.
7. If needed, browse to select a folder for your exported trace data in the **Export Path** field. This directory path is automatically saved until changed. The default path setting is **C:\Users\AdminUser\Documents\Molecular Devices\IonWorks Barracuda\Export**.

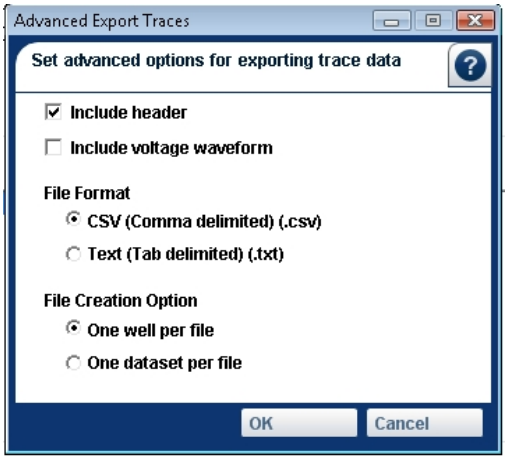
   Note: If you have an invalid **Export Path** entered, such as to a USB flash drive that has been removed, you will be prompted to change the **Export Path** entry to an existing directory.
8. Type a folder name for the exported trace data files in the **Traces Folder Name** field. IonWorks Barracuda® System Software automatically creates numbered folders for each well within the designated folder.
9. Click **Export** to export the selected trace data, or click **Advanced** to set additional

options.



To set Advanced options for exporting trace data:

1. Follow the instructions for exporting trace data, then click **Advanced**. The Advanced Export Trace Data dialog opens.



2. Select the **Include header** check box to include header data such as scan type and parameter values.
3. Select a file format from the **File Format** field: CSV for comma-delimited data, or TXT for tab-delimited data.
4. Select an option from the **File Creation Option** field: One well per file, or one data set (scan) per file.

5. Click **OK** to close the Advanced Export Traces dialog and return to the Export Traces dialog.
6. Click **Export** to immediately export the selected trace data.
7. To view the exported data, click **File > Open Exported Data Folder** and select from the list of exported data files in the folder.

Note: If you have an invalid **Export Path** entered, such as to a USB flash drive that has been removed, the software automatically exports the data to the default location **C:\Users\AdminUser\Documents\Molecular Devices\IonWorks Barracuda\Export**.

Chapter 14: Completing Instrument Cleanup

14

The most important step in cleaning up for the day is to run the **End of Day Flush and Rinse**. See Performing the End of Day Flush and Rinse on page 149.

The following overview describes general procedures for instrument cleanup:

1. After the experiment is completed, discard unused solutions and rinse the cell boat, and buffer boat with a 50% Ethyl Alcohol (EtOH) solution followed by DI water.

   Note: Leave the last PatchPlate in place to use for the next **Start of Day Flush and Rinse** run.

2. Unload the pipettor tips. You can do this manually using instrument utilities, or you can include this step in the Cleanup Protocol using the **Discard Tips** option. Skip this step if you are planning to re-use the tips.

   Note: Tips can be unloaded from the pipettor head and reloaded approximately nine times before you must replace the tip rack with new tips.

3. Empty all of the fluid bottles under the process deck, except the Ethyl Alcohol bottle. See The Fluid Handling Components on page 40.
4. Run **Utilities > End of Day Flush and Rinse**. See Performing the End of Day Flush and Rinse on page 149.
5. Unload the CellPettor pipette.
6. Empty the waste carboys. The waste carboys must be emptied daily, either at the end of the day or before the next day's operations. See Emptying Waste Carboys on page 102.
7. Check the overflow reservoir (sink) in the lower section of the instrument cabinet to the right of the source carboys. The overflow reservoir is a catch for any spills that might occur at the PatchPlate station or on the process deck. The capacity of the reservoir is limited, so if any fluid is present, sponge the liquid out to prevent overflow of the reservoir. If liquid is present, but you do not recall any spills, contact Molecular Devices Technical Support.

Performing the End of Day Flush and Rinse

The most important step in cleaning up for the day is to run **End of Day Flush and Rinse**.

Note: Make sure you leave in place a CellPettor aspirating pipette and a clean, undamaged PatchPlate while **End of Day Flush and Rinse** runs.

To perform the End of Day Flush and Rinse:

1. Make sure the Alcohol bottle is filled with at least 100 mL of 50% Ethyl Alcohol (EtOH).
2. Empty all of the fluid bottles under the process deck, except the Ethyl Alcohol bottle. See The Fluid Handling Components on page 40.
3. In the shortcut bar, click  , and then click **End of Day Flush and Rinse**. The instrument door unlocks.
4. Make sure that a clean PatchPlate is loaded at the PatchPlate station and that the CellPettor has an aspirating pipette loaded.

   CAUTION! A clean PatchPlate can be reused as long as it is in good condition with no damage to any part of the consumable. Using a delaminated, chipped, marred, or misshapen PatchPlate could result in damage to the Electrode-Plate, or instrument errors.

5. Click **Continue**. The **End of Day Flush and Rinse** operation runs for approximately five minutes and sterilizes the equipment for the next day.
6. Empty the waste carboys after **End of Day Flush and Rinse** completes. See Emptying Waste Carboys on page 102.

CAUTION! To maintain the system properly, you must run **End of Day Flush and Rinse** within six days of system usage. Otherwise, on the seventh day, the system with stop running experiments until you run **End of Day Flush and Rinse**.

Unloading the CellPettor Pipette

To remove the pipette from the CellPettor:

1. In the software, click **Instrument > Utilities**. The **Manual Instrument Control** dialog appears.
2. Click **Unload CellPettor Pipette**. The instrument pauses operations and unlocks the door to the process deck.
3. While firmly holding the cylindrical mounting tube on the CellPettor, pull down to loosen and remove the aspirating pipette.
4. Replace the pipette, as needed.

Chapter 15: Maintaining the Instrument

15

The IonWorks Barracuda System requires daily maintenance of the fluid-handling system with the Flush and Rinse procedures. The instrument surfaces should be kept clean and dry with extra care applied to the plenum surface and the Electrode-Plate.

For instructions on the Flush and Rinse procedures, see:

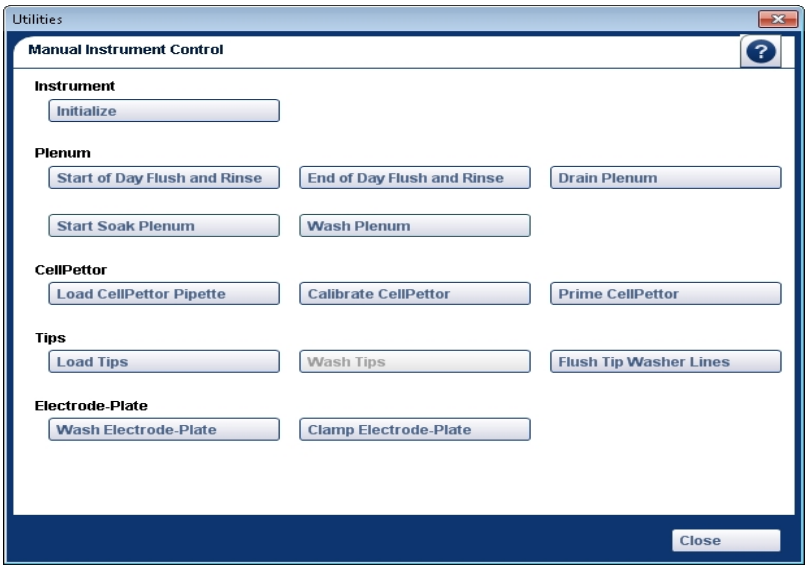
- Performing the Start of Day Flush and Rinse on page 105
- Performing the End of Day Flush and Rinse on page 149

Using Manual Instrument Controls

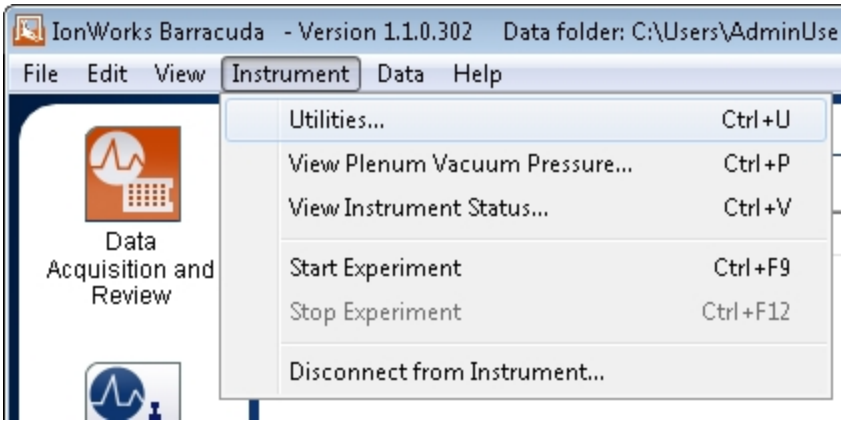
IonWorks Barracuda® System Software includes utilities that can be used to manually control some functions of the instrument.

To open the **Manual Instrument Control** window:

- From the shortcut toolbar, click  . The **Utilities** dialog appears.



- Alternatively, click **Instrument > Utilities**.



The most commonly used utilities for maintaining the instrument include the **Start of Day Flush and Rinse** and the **End of Day Flush and Rinse**.

To use these utilities, see:

- Performing the Start of Day Flush and Rinse on page 105
- Performing the End of Day Flush and Rinse on page 149

Other commonly used utilities include:

- Loading the CellPettor Pipette on page 112 or Unloading the CellPettor Pipette on page 150
- Loading the Tip Rack on page 106
- Flushing the Tip Washer Lines on page 165

If the instrument enters a **Fault** state, you might need to use the **Initialize** utility. **Initialize** cancels any operation in progress, drains any fluids, if applicable, and re-calibrates all components except the CellPettor. For more information, see Initializing the Instrument on page 169.

The remaining utilities you might need to use occasionally, include:

- **Drain Plenum**
- **Start Soak Plenum** or **End Soak Plenum** (see Soaking the Plenum Manually on page 167)
- **Wash Plenum** (see Washing the Plenum on page 166)
- **Calibrate CellPettor** (see Calibrating CellPettor on page 182)
- **Prime CellPettor**
- **Wash Electrode-Plate** (see Step 1: Specify Instrument Cleanup on page 97)
- **Clamp Electrode-Plate** or **Unclamp Electrode-Plate**

The instrument generally performs several of the less needed utility operations automatically during normal use, either during the Flush and Rinse processes, or during assays. Several of the less needed utility operations are provided in case of power outages or unforseen circumstances that might require manual instrument controls.

Disinfecting the Process Deck

The surface of the process deck should be periodically disinfected by wiping with 70% isopropyl alcohol using a lint-free cloth. Follow this step by wiping again with 50% alcohol (EtOH) using a lint-free cloth.

The glass door can be cleaned with standard glass cleaner.

Conditioning Replaceable Ground Electrodes (REGEs)

Note: This procedure does not apply to instruments equipped with the standard plenum. See Plenum on page 34.

The purpose of the conditioning process is to equilibrate the REGE set to the internal buffer that you will use in your next assay.

Before installation and use, the REGE set must be conditioned in the correct internal buffer. For ideal equipment maintenance purposes, Molecular Devices recommends also conditioning each REGE set at the end of the usage day using fresh internal buffer overnight.

After conditioning, the REGEs go back in to the IonWorks Barracuda PatchPlate station REGE plenum sockets to continue running assays, or should be stored dry in the original packaging.

REGEs stay conditioned as long as they are unused, untouched by bare hands, and properly equilibrated.

CAUTION! Never touch the top surface of the ground electrodes with your bare hands, because it results in a build-up of oils on the electrodes that impairs the functionality.

To condition a REGE set:

1. Fill the included deep buffer boat with the same kind of internal buffer that you will use for your next assay.
2. Place the four REGEs upside-down in the buffer boat with the electrode heads on the bottom of the buffer boat.
3. Add enough additional internal buffer fluid, as needed, until the electrodes are completely submerged.

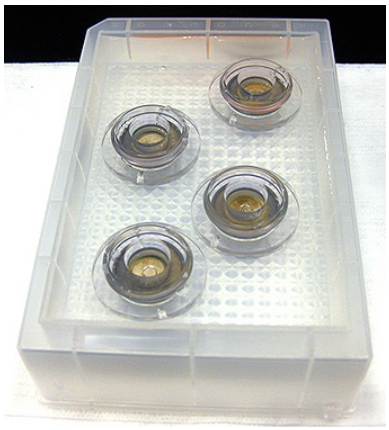


Figure 15-1: REGE Set Soaking in Internal Buffer

4. Remove air bubbles trapped against the electrodes by gently tapping the submerged housings.
5. Soak overnight. Molecular Devices recommends between 13 to 15 hours.
6. After soaking the electrodes overnight, wearing gloves, remove the REGEs from the buffer boat, and rinse each briefly over a sink with distilled water.
7. Dry the REGE housing, especially the underside.

CAUTION! If not removed from the underside of the REGE housing, the salt in the internal buffer accumulates in the REGE plenum socket, which can cause an open circuit when the salt dries.

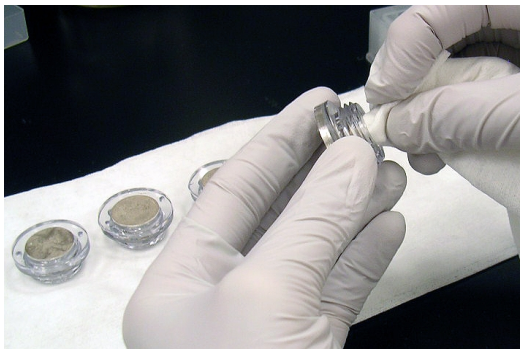


Figure 15-2: REGE Housing Underside Drying

8. Install the REGEs in the IonWorks Barracuda instrument plenum to continue running assays. See Installing Replaceable Ground Electrodes (REGEs) on page 155.

9. After conditioning, unused REGEs should be stored dry in the original packaging with the lid closed.

CAUTION! Once your REGEs are dry, if you are going to immediately use them to run assays, do not rinse the electrode heads with distilled water, because your assays can experience voltage drift while the chloride concentration in the electrodes equilibrates to the chloride in the internal buffer
When completely dry, the REGEs rapidly absorb approximately 200 µL of the liquid that first wets them, and it takes about 90 minutes for the REGEs to equilibrate to a different buffer.

Installing Replaceable Ground Electrodes (REGEs)

Note: This procedure does not apply to instruments equipped with the standard plenum. See Plenum on page 34.

Only install REGE sets that have been properly conditioned. See Conditioning Replaceable Ground Electrodes (REGEs) on page 153.

CAUTION! Always wear gloves when working within the IonWorks Barracuda instrument and handling the REGE parts. Never touch the top surface of the ground electrodes with your bare hands, because it results in a build-up of oils on the electrodes that impairs the functionality.

To install the REGEs in the plenum:

1. Verify that the O-ring and spring are correctly positioned, and correct as needed.
   The O-ring should be seated in the designated O-ring counter-bore, and the spring should be over the plug in the center of the socket with the narrow diameter of the spring at the top.

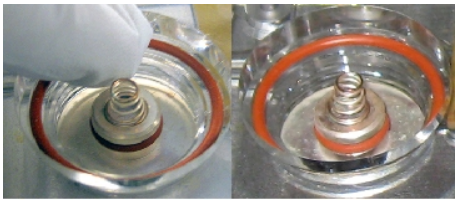


Figure 15-3: Correctly Positioned O-Ring and Spring in Empty Plenum Socket

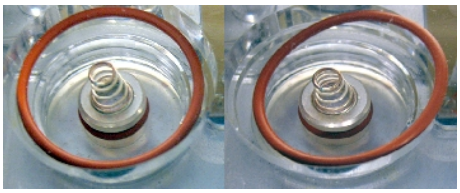


Figure 15-4: Incorrectly Positioned O-Ring and Spring in Empty Plenum Socket

2. Verify that the plenum socket is dry. Depending on the amount of liquid in the socket, use a cotton swab or wipe cloth.

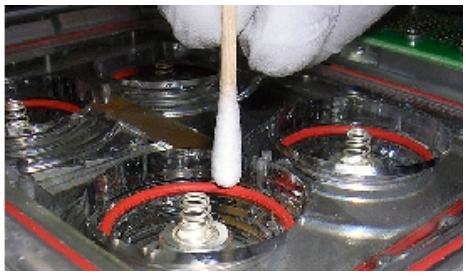


Figure 15-5: REGE Plenum Drying

3. Wet the REGE housing screw threads with 10 µl to 20 µl distilled (DI) water for lubrication. The amount of DI water depends on how long your REGE set has been dry.

- If you are installing immediately after conditioning, when drying the housing, leave 20 µl of the DI water rinse on the threads.
- If you are installing when the REGE set is completely dry, lubricate the housing threads with 10 µl of DI water, rotate the housing 180 degrees, and apply 10 µl of DI water to the threads again.

Figure 15-6: REGE Housing Threads Lubrication with 20 µl Distilled Water

4. Insert the lubricated REGE loosely into an empty plenum electrode socket.

Figure 15-7: REGE Placement in a Plenum Socket

Note: Try not to dislodge the spring and O-ring inside the plenum socket. If the spring and O-ring inside the socket become dislodged, put them back in place.

5. Using the provided REGE service tool, seat the tool prongs into the holes in the electrode housing for installation.

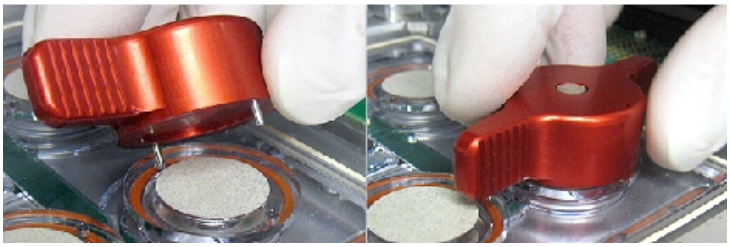


Figure 15-8: REGE Service Tool Prongs Align with Holes in the Electrode Housing

6. Turn counterclockwise a ¼ turn to align the male and female threads and reduce the risk of cross-threading.
7. Place your index finger on one hand on the magnet on the top of the service tool and apply downward pressure while using your other hand to turn the service tool clockwise to keep the service tool pins in the electrode housing service tool holes.

Figure 15-9: Two Hand Usage of the REGE Service Tool

8. Turn until the electrode housing seats in the plenum socket flush with the plenum top.
9. Run a gloved finger along the seam between the REGE and plenum to confirm a flush installation.

   The two surfaces should be smooth and bump-free.

Figure 15-10: Gloved Finger Flush Installation Test

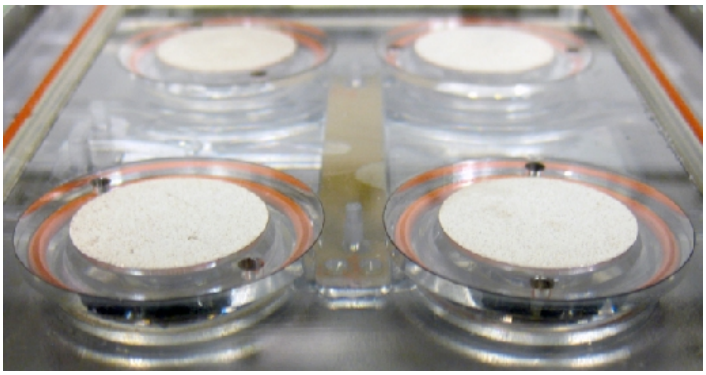


Figure 15-11: Correct Flush REGE Installation

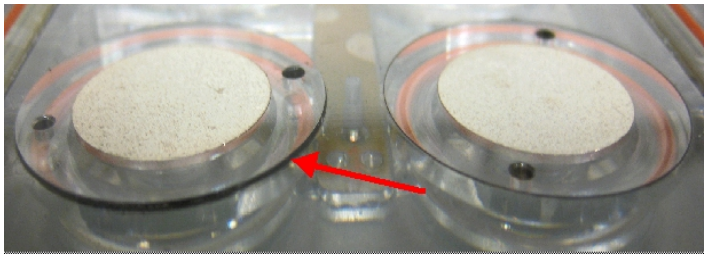


Figure 15-12: Incorrect Raised REGE Installation

10. Repeat steps 1-7 until all four new ground electrodes have been added to the plenum.
11. After all four REGEs are properly installed in the plenum, you can begin your assays.

CAUTION! Never touch the ground electrode top surface with your bare hands, because it results in a build-up of oils on the electrodes that impairs the functionality.

Removing Replaceable Ground Electrodes (REGEs)

Note: This procedure does not apply to instruments equipped with the standard plenum. See Plenum on page 34.

Remove and condition each REGE set nightly after use. You reuse the REGEs that you remove, so remove them carefully. Always wear gloves when working on the IonWorks Barracuda instrument and handling the REGE parts.

CAUTION! Always wear gloves when working within the IonWorks Barracuda instrument and handling the REGE parts. Never touch the top surface of the ground electrodes with your bare hands, because it results in a build-up of oils on the electrodes that impairs the functionality.

To remove the REGEs in the plenum:

1. Using the provided REGE service tool, seat the tool prongs into the holes in the sides of the electrode housing for removal.

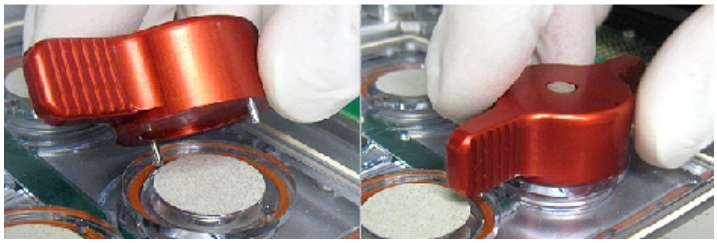


Figure 15-13: REGE Service Tool Prongs Align with Holes in the Electrode Housing

2. To keep the service tool pins in the electrode housing service tool holes, place your index finger on one hand on the magnet on the top of the service tool, and apply downward pressure while using your other hand to turn the service tool counterclockwise until the REGE housing unscrews and is loose in the plenum socket.

Figure 15-14: Two Hand Usage of the REGE Service Tool

3. Lift the REGE housing out of the plenum socket and carefully set it aside for conditioning

Figure 15-15: REGE Removal from a Plenum Socket

Note: Try not to dislodge the spring and O-ring inside the plenum socket. If the spring and O-ring inside the socket become dislodged, put them back in place.

4. Dry the plenum socket.

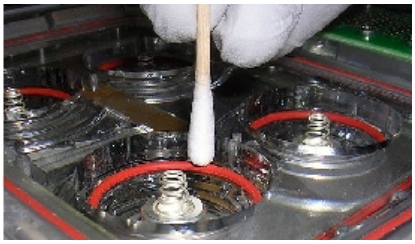


Figure 15-16: REGE Plenum Drying

5. Repeat steps 1-4 until all four REGEs have been removed from the four plenum sockets.
6. Condition all four REGEs to continue using them in the IonWorks Barracuda system. See Conditioning Replaceable Ground Electrodes (REGEs) on page 153

Note: Proper conditioning takes up to 15 hours.

7. To continue using the IonWorks Barracuda system while the recently removed REGE set conditions over night, install the provided second REGE set. See Installing Replaceable Ground Electrodes (REGEs) on page 155, otherwise, place a written note over the plenum that reads something like “Electrodes Have Been Removed” as a precautionary reminder to keep the plenum dry.

Rechloriding the Electrode-Plate

As a guideline, Molecular Devices recommends rechloriding the Electrode-Plate electrode pins when the Standard Deviation (SD) of the hole test offset consistently exceeds 4 mV in three or four successive experiment runs. A periodic spike over 4 mV does not warrant rechloriding.

Review the median voltage offset values for the Hole Test Pre-correction scan. Values falling outside the median range of ±20 mV likely indicate the need for rechloriding. Monitor your results to determine whether your electrode pins need rechloriding.

CAUTION! To avoid damage to the Electrode-Plate:

- Do not touch the electrode pins or the pogo pins on the underside of the Electrode-Plate.
- Keep the pogo pins dry.
- Never immerse the Electrode-Plate in liquid of any kind. The electrode pins, however, can be immersed in a bleach solution for rechloriding.
- Always lift the Electrode-Plate using the handles on the top.
- Always set the Electrode-Plate on a flat surface resting on its four stand-offs or on the Bleach Boat.

To rechloride the Electrode-Plate electrode pins:

1. Fill the Rechloriding Bleach Boat reservoir with enough reagent-grade 50% Sodium Hypochlorite (use Sigma Aldrich, PN 425044-1L) and 50% DI (deionized) water solution to fill the reservoir.

   CAUTION! Do not use household bleach to rechloride the Electrode-Plate. Household bleach can damage the electrodes.

2. Lift the Electrode-Plate using the handles on the top to avoid damage.
3. Set the Electrode-Plate onto the bleach boat with the stand-offs positioned appropriately so that the Electrode-Plate fits correctly into the molded surface. Make sure the electrode pins are submerged in the solution.
4. Soak the electrode pins for approximately 15 minutes.

   CAUTION! Do not over-chloride as damage to the electrodes can occur.

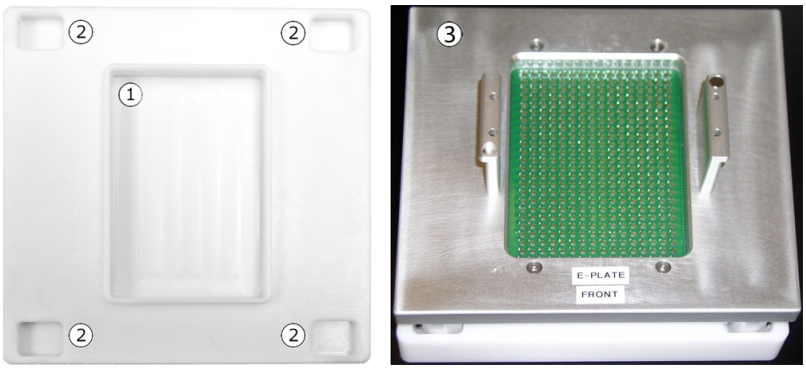


Figure 15-17: Bleach Boat and Electrode-Plate

Component	Description
1	Rechloriding Bleach Boat reservoir
2	Indentations for Electrode-Plate standoffs
3	Electrode-Plate on Bleach Boat

Cleaning the Amplifier Touch Pads

Ideally, the amplifier touch pads on the sides of the plenum should never be touched, nor should the corresponding pogo pins on the Electrode-Plate and Model Cell. Oils on the touch pads and pogo pins can degrade the signal quality. If any degradation, such as an open circuit, is detected, the touch pads and pogo pins can be cleaned very gently with distilled water, isopropyl alcohol, and a lint-free cloth.

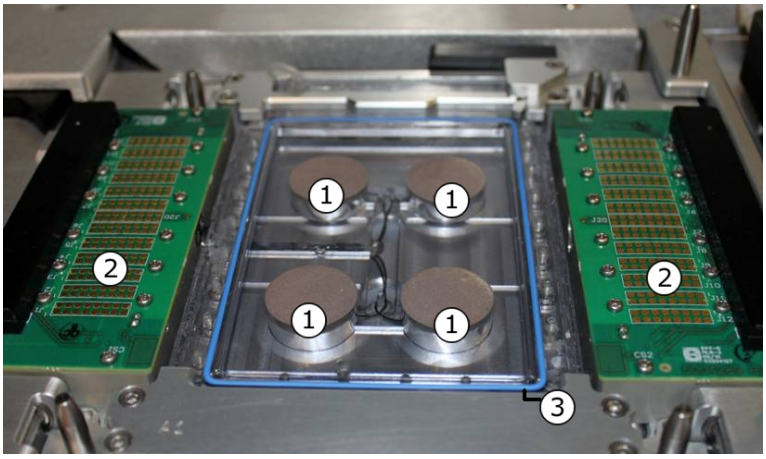


Figure 15-18: The Amplifier

Position	Component
1	Plenum ground electrodes
2	Amplifier touch pads
3	Plenum O-ring (rubber gasket). The color of the O-ring might vary.

To clean the amplifier touch pads:

1. Moisten a lint-free cloth with distilled water, and wipe off any salt deposit buildup contaminants on the amplifier touch pads.
2. Moisten a lint-free cloth with isopropyl alcohol, and wipe off any oily buildup contaminants on the amplifier touch pads.

   CAUTION! Never pour any liquids directly on to the PCBs. Doing so can damage the circuitry.

3. Let the cleaned amplifier touch pads dry completely before continuing.

   CAUTION! Before retesting **Instrument Status**, to avoid damage, make sure all of the printed circuit boards (PCBs) on the touch pads and Electrode-Plate, or Model Cell are completely dry.

4. Run an assay. If any of the wells in the PatchPlate show errors, try cleaning the touch pads again.
   If the problem persists, the amplifier pogo pins might be dirty. See Cleaning the Amplifier Pogo Pins on page 162.

For amplifier touch pad cleaning problems, contact Molecular Devices Technical Support.

Cleaning the Amplifier Pogo Pins

The pogo pins can be cleaned very gently with distilled water, isopropyl alcohol, and a lint-free cloth, if any degradation, such as an open circuit, is detected. See Cleaning the Amplifier Touch Pads on page 161.

Note: The amplifier touch pads on the sides of the plenum should never be touched, nor should the corresponding pogo pins on the Electrode-Plate and Model Cell, because oils on the touch pads and pogo pins can degrade the signal quality.

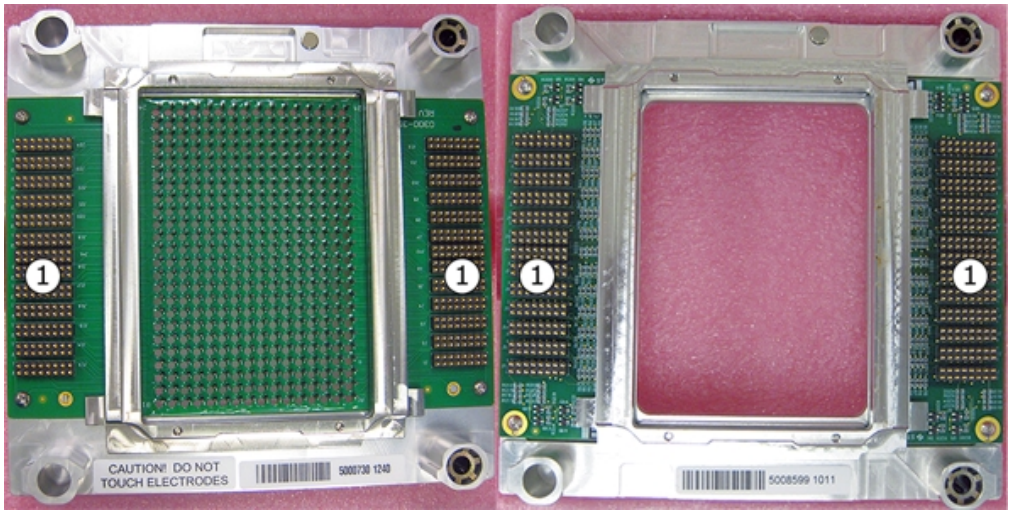


Figure 15-19: Back of the Standard Electrode-Plate and Model Cell

Position	Component
1	Pogo pins (connect with amplifier touch pads)

To clean the pogo pins:

1. Lift the Electrode-Plate or Model Cell off the process deck and place it on a safe surface upside-down.
2. Moisten a lint-free cloth with distilled water, and wipe off any salt deposit buildup contaminants on the amplifier touch pads.

   CAUTION! Never pour any liquids directly on to the PCBs. Doing so can damage the circuitry.

3. Moisten a lint-free cloth with isopropyl alcohol, and wipe off any oily buildup contaminants on the amplifier touch pads.

   CAUTION! Make sure you remove all cloth fiber debris left during your wipe.

4. Let the cleaned Electrode-Plate or Model Cell dry completely before placing it on the process deck again.
5. Verify cleaning success in the **Instrument Status** dialog. See Viewing Instrument Status on page 172.

For pogo pin cleaning problems, contact Molecular Devices Technical Support.

Cleaning the Electrode-Plate and Model Cell

When an Electrode-Plate or Model Cell is on the PatchPlate station, the capacitance sensors determine its presence by measuring the generated capacitance of the metal tabs on the underside of an Electrode-Plate and Model Cell being close to the sensing element in the amplifier touch pad boards. The presence of the Electrode-Plate or Model Cell registers in the **Instrument Status** dialog.

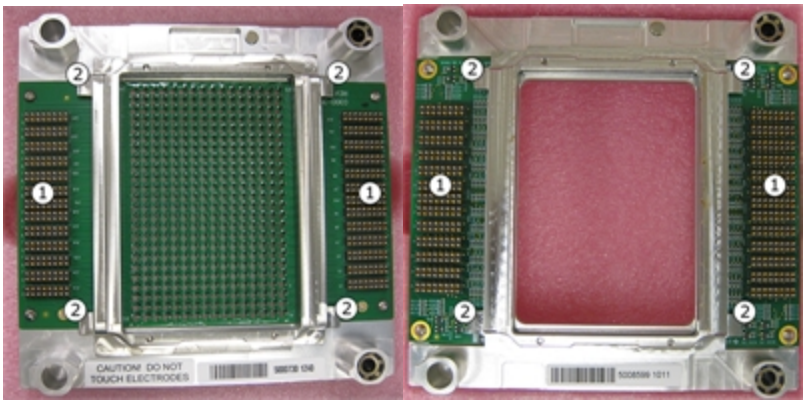


Figure 15-20: Underside of the Standard Electrode-Plate and the Model Cell

Position	Component
1	Pogo pins (connect with amplifier touch pads)
2	Capacitive metal tabs (hover above capacitance sensors)

The metal tabs on the underside of the Electrode-Plate and Model Cell, as well as the corresponding sensors, can be exposed to liquids and salt solutions contamination during experiments. Contamination can cause an **Electrode-Plate is tilted** error message to appear in the IonWorks Barracuda® System Software.

When the error message appears, you need to verify that the Electrode-Plate or Model Cell is correctly placed on the PatchPlate Station, and then clean the capacitive metal tabs on the Electrode-Plate or Model Cell, depending on which you are using at the time of the error message appearance. You should also clean the capacitance sensors on the amplifier touch pad. See Cleaning the Capacitance Sensors on page 179.

CAUTION! Always wear gloves when touching parts of the process deck, including an Electrode-Plate or Model Cell. Oils on your hands can damage the equipment and the fluids used for the experiments are hazardous.

To clean the Electrode-Plate or Model Cell:

1. Lift the Electrode-Plate or Model Cell off the process deck and place it on a safe surface upside-down.
2. Moisten a lint-free cloth with distilled water, and wipe off any salt deposit contaminants that built-up on the capacitive metal tabs.
3. Moisten a lint-free cloth with isopropyl alcohol, and wipe off any oily contaminants that built-up on the capacitive metal tabs.

   CAUTION! Never pour any liquids directly on to the PCBs. Doing so can damage the circuitry.

4. Let the cleaned Electrode-Plate or Model Cell dry completely before replacing it on the process deck.

   Before retesting **Instrument Status**, to avoid damage, make sure all of the printed circuit boards (PCBs) on the touch pads and Electrode-Plate, or Model Cell are completely dry.
5. Verify cleaning success in the **Instrument Status** dialog. See Viewing Instrument Status on page 172.

   Errors reported in the **Instrument Status** dialog require cleaning the capacitance sensors. See Cleaning the Capacitance Sensors on page 179.

For Electrode-Plate or Model Cell cleaning problems, contact Molecular Devices Technical Support.

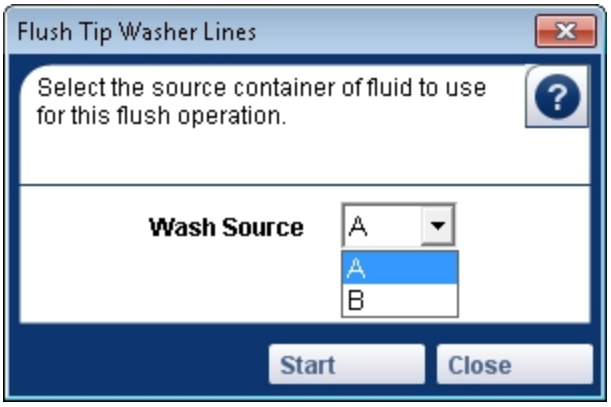
Flushing the Tip Washer Lines

The **Flush Tip Washer Lines** utility is an instrument maintenance process that runs bleach through the lines to clean out growths and debris that can develop in the tubes, which cause blockages.

Note: Molecular Devices recommends running this process once every three months.

To run Flush Tip Washer Lines:

1. Replace the contents of your Wash Source bottle, A or B, with 500 mL of 2% bleach, and empty both of the Waste bottles. See The Fluid Handling Components on page 40 and Filling Source Bottles with Wash Solutions on page 104.
2. In the shortcut bar, click , and then click **Flush Tip Washer Lines**. The **Flush Tip Washer Lines** dialog appears.



3. In the **Wash Source** field, select **A** or **B** according to the bottle you filled with bleach solution in step 1.
4. Click **Start** to begin.

   A one-time instructional message dialog appears explaining Steps 5 through 8.
5. Click **Continue**.

   The process ends when the bleach solution runs out.
6. Rinse the used wash source bottle, A or B, with Deionized (DI) water.
7. Replace the contents of the used wash source bottle, A or B, with 500 mL of DI water.
8. Click **Start** to flush residual bleach solution out and to prime the lines.

   Note: Note: Failure to flush and prime the lines with DI water (Steps 5 through 8) can cause your experiments to fail.
9. Before you start your experiment, run **Start of Day Flush and Rinse**. See Performing the Start of Day Flush and Rinse on page 105.

Washing the Plenum

Note: This procedure is specifically for use with instruments equipped with the standard plenum containing built-in ground electrodes.

The **Wash Plenum** utility automatically runs up to five soak cycles. Each soak cycle involves filling the plenum with internal buffer fluid, soaking the plenum as specified, draining the internal buffer fluid at the end of the soak duration, and repeating the soak cycle as specified.

CAUTION! Do not use **Wash Plenum** if your instrument is equipped with the replaceable ground electrodes (REGEs) plenum, especially if the REGEs have been removed from the plenum for conditioning.

Wash Plenum can be run between experiments or overnight.

Note: If running overnight, Molecular Devices recommends first cleaning up the instrument as you would at the end of the day. See Completing Instrument Cleanup on page 149.

To run Wash Plenum:

1. Make sure there is 50 mL of internal buffer fluid in the internal buffer bottle per soak cycle, and refill the internal buffer bottle, as needed. See The Fluid Handling Components on page 40 and Filling Buffer, Alcohol, and Cell Perforation Agent Bottles on page 103.
2. In the shortcut bar, click  , and then click **Wash Plenum**. The **Wash Plenum Settings** dialog appears.
3. In the **Soak Duration** field, type the soak time in minutes (maximum 1440 minutes/24 hours).
4. In the **Number of cycles** field, type the number of times the soak cycle repeats (maximum 5 times).
5. Click **Start** to begin.

Soaking the Plenum Manually

Note: This procedure is specifically for use with instruments equipped with the standard plenum containing built-in ground electrodes.

The plenum can be soaked manually for an extended period of time using the **Start Soak Plenum** utility. Soaking the plenum uses internal buffer fluid.

Soaking the plenum manually for an extended period of time can be done between experiments.

CAUTION! Do not use **Start Soak Plenum** if your instrument is equipped with the replaceable ground electrodes (REGEs) plenum, especially if the REGEs have been removed from the plenum for conditioning.

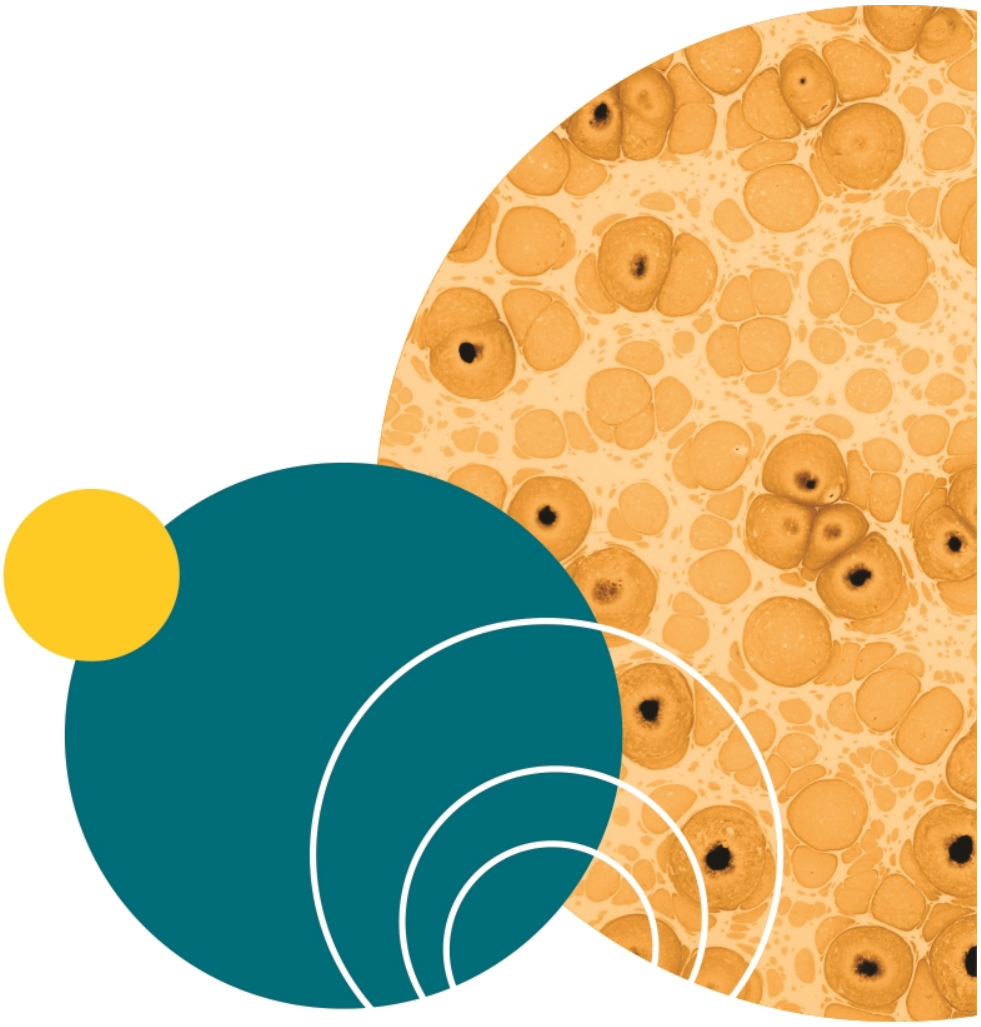
To soak the plenum manually for an extended period of time:

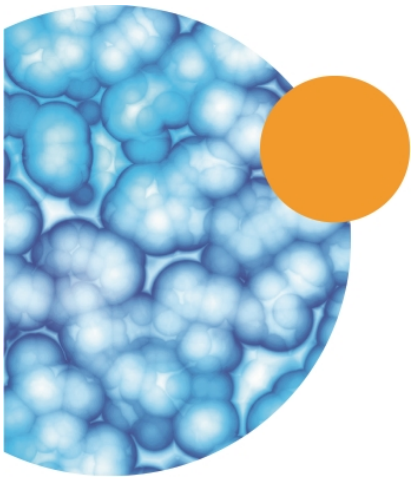
1. Make sure there is approximately 50 mL of internal buffer fluid in the internal buffer bottle, and refill the internal buffer bottle, as needed. See The Fluid Handling Components on page 40 and Filling Buffer, Alcohol, and Cell Perforation Agent Bottles on page 103.
2. In the shortcut bar, click  , and then click **Start Soak Plenum**.
3. To finish soaking the plenum, click  , and then click **End Soak Plenum** to drain the internal buffer fluid.

Soaking the plenum overnight should be done after the instrument cleanup. See Completing Instrument Cleanup on page 149.

To soak the plenum overnight:

1. Run the **End of Day Flush and Rinse** cleanup process. See Performing the End of Day Flush and Rinse on page 149.
2. Refill the internal buffer bottle, as needed. See The Fluid Handling Components on page 40 and Filling Buffer, Alcohol, and Cell Perforation Agent Bottles on page 103.
3. In the shortcut bar, click  , and then click **Start Soak Plenum**.
4. To finish soaking the plenum, click  , and then click **End Soak Plenum** to drain the internal buffer fluid.





Chapter 16: Troubleshooting

16

The Automated Patch Clamp System is designed to operate using sensors to monitor the status of the instrument at all times. If the system displays an error, and you are not sure how to respond, please contact Molecular Devices so that we can assist you.

Technical Assistance

There are several ways to contact Molecular Devices:

- World Wide Web: http://www.moleculardevices.com
- Phone: +1 (800) 635-5577
- Fax: +1 (408) 747-3603
- User Assistance: See the Molecular Devices Knowledge Base at http://www.moleculardevices.com/Support.html

Initializing the Instrument

During the initialization process, the software ensures that the instrument is ready for the next experiment by recalibrating all of its subsystems. During initialization, the software confirms that instrument components are properly positioned, used fluids are pumped out, new fluids are available, and sensor readings are all within acceptable levels.

As the IonWorks Barracuda System is running, it operates in any one of several instrument "states." These states appear in the status bar at the bottom of the main software window.

- **Ready:** All components are initialized and waiting for instructions.
- **Running:** The instrument is performing a normal operation.
- **Initializing:** The instrument is performing automated calibrations and fluid-handling tasks to prepare for use.
- **Paused:** The user has asked the system to wait until it is signaled to continue.
- **Idle:** The instrument has received no commands for several minutes and is in power-saving mode.
- **Fault:** The instrument cannot continue with normal operations and must be re-initialized.

Occasionally, an error might occur which interrupts normal operations such as an obstacle on the process deck or an empty external buffer boat. These errors might cause a Fault state and must be followed by initializing the instrument.

To initialize:

1. In the software menu, click **Instrument > Utilities > Initialize**. The instrument cancels any operation in progress, drains any fluids if applicable, and re-calibrates all components except the Cellpettor.
2. After initializing, the door unlocks so that you can clear any unusable cell suspension, compound plates, the PatchPlate, or other used labware from the process deck.
3. Replace the necessary buffer solution and compound plates, and insert a new PatchPlate. Close the door when this step is complete.
4. After the door locks, the instrument returns to the Ready state and you can begin a new experiment.

Using the Model Cell to Diagnose Signal Issues

The Model Cell mimics a whole cell patch clamp assembly using resistors, and can be used in place of the Electrode-Plate for any protocol. By removing the variability of a fluid and cell environment, the Model Cell can also serve as a diagnostic tool for testing the integrity of the Patch Engine components including electrodes, pogo pins, and amplifiers.

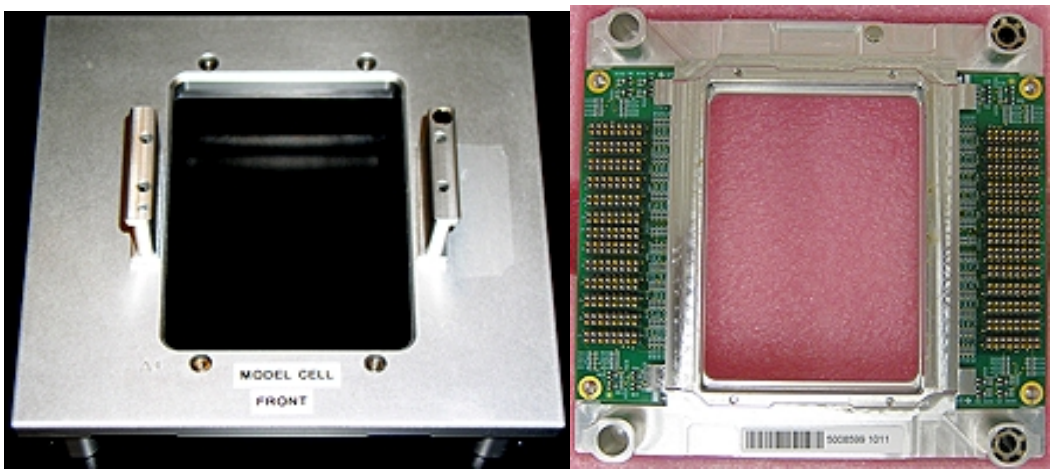


Figure 16-1: Front and Back of the Model Cell

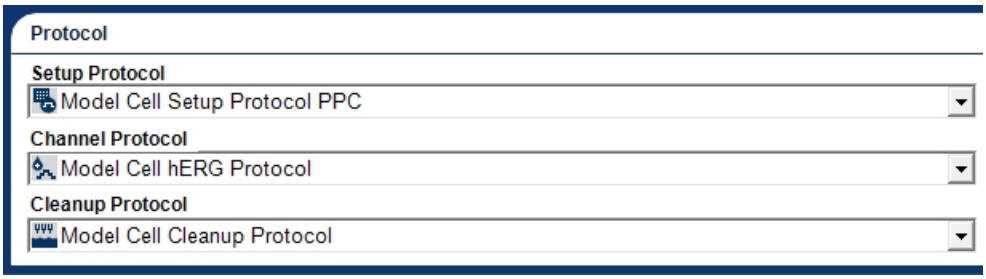
The Model Cell is divided into two sides:

- The left bank of resistors (**A1**:**H24**) mimics a population patch clamp (PPC) PatchPlate at 200 kΩ. Fifty nano-amps (50 nA) of current is expected for every 10 mV applied in the protocol. Keep in mind that the currents for this bank easily reach the instrument limit (110 nA) for protocols run in single-hole (SH) mode.
- The right bank of resistors (**I1**:**P24**) mimics a single-hole (SH) PatchPlate at 10 MΩ. One nano-amp (1 nA) of current is expected for every 10 mV applied in the protocol.

When you review the data from a Model Cell assay, keep in mind that the two types of resistors produce different results, some of which might not apply to your intended test.

To use the Model Cell to diagnose signal issues:

1. In the Protocol area of the Data Acquisition and Review workspace, select the Model Cell protocols you want to use.



2. Place the Model Cell unit on the wash station in place of the Electrode-Plate.
3. If you want to adjust the voltage parameters, start the experiment in **Assay Development Mode**.
4. To switch between SH and PPC testing, change the **Setup Protocol** > **PatchPlate Type** selection , then run the assay again. Expect current saturation in all wells served by the PPC resistor bank when running a SH protocol and applying voltage less than –25 mV or greater than +25 mV.

Errors

You can encounter various instrument component sensors that cause Patch Engine errors. The following are solutions to the most common errors.

Error	Verification	Solution
The Electrode-Plate is Tilted	Testing Patch Engine Sensor Status on page 173	Cleaning the Capacitance Sensors on page 179 then do verification again
The PatchPlate is Tilted	Testing Patch Engine Sensor Status on page 173	Cleaning the Amplifier Optical Sensors on page 181 then do verification again
Same Wells Fail Every Time	Run an assay, as needed	Cleaning the Amplifier Touch Pads on page 161 then do verification again
Amphotericin Tubing Leaks	Finding an Amphotericin Pump Tube Leak on page 182	Replacing the Amphotericin Pump Insert Tube on page 183

Viewing Instrument Status

The **Instrument Status** dialog allows you to view the current status of the various instrument component sensors.

To view the **Instrument Status** dialog, click **Instrument** > **View Instrument Status**.

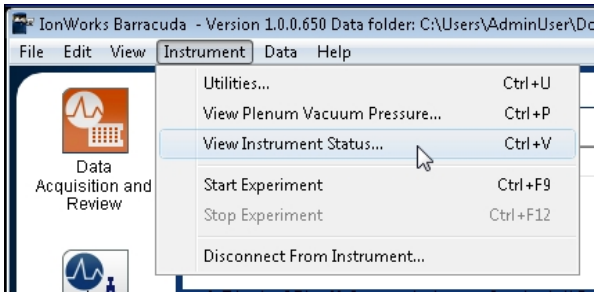


Figure 16-2: Instrument Menu

A list of each of the instrument components with the status of the most recent instrument sensor test appears in the **Instrument Status** dialog.

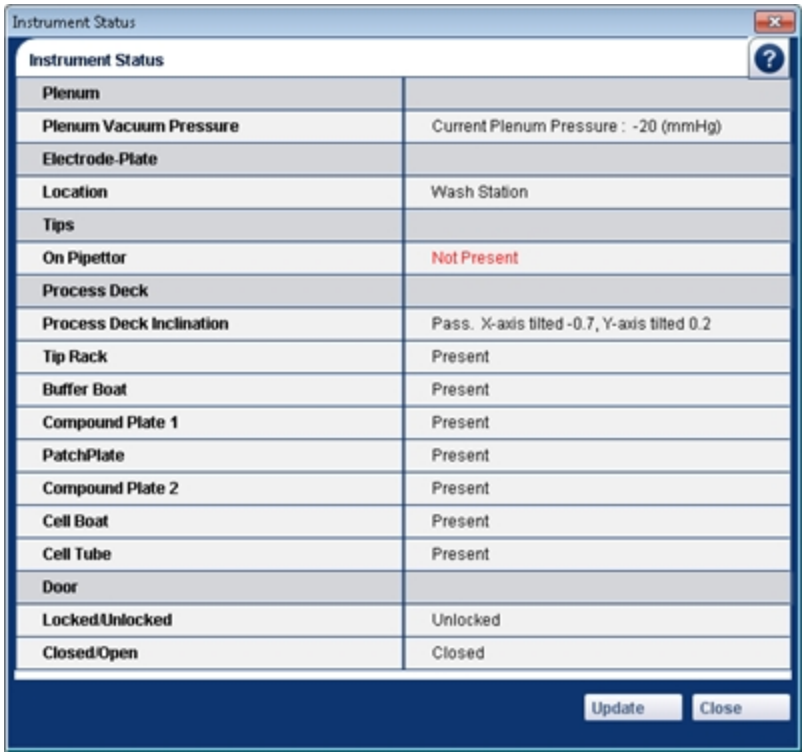


Figure 16-3: Instrument Status Dialog

Status results indicate whether you need to take any corrective action. The status results vary according to the instrument component and the associated sensor purpose, such as **Tips On Pipettor** | **Not Present** or **Electrode-Plate Location** | **Wash Station**.

Note: If the component status **Process Deck Inclination** | **Fail** appears, and your assay performance is negatively impacted, contact Molecular Devices for technical support.

To refresh the information after doing something that changes a component status, such as placing the Electrode-Plate on the PatchPlate station, click **Update** to view the new status results.

To verify that the Patch Engine sensors are functioning properly, there are five Patch Engine sensor tests that you run while the **Instrument Status** dialog is open in the software. Molecular Devices recommends running all of these tests every time you clean parts of the Patch Engine. See Testing Patch Engine Sensor Status on page 173.

Testing Patch Engine Sensor Status

There are five **Instrument Status** tests you run to verify that the Patch Engine sensors are functioning properly. These tests involve the PatchPlate station, a PatchPlate, a Model Cell, and an Electrode-Plate on the process deck, and the **Instrument Status** dialog open in the software. The status results indicate whether you need to take any corrective action.

Complete all five tests when verifying Patch Engine sensor status.

- If all of the tests indicate correct sensor statuses, the sensors are all functioning properly.
- If any of these status verification tests indicate corrective action, after completing the specified corrective action, run all four verification tests again to confirm the success of the corrective action.

The following is the order that verification testing should be done:

1. Test with nothing on the PatchPlate station. See Patch Engine Sensor Test 1 on page 174.
2. Test with only the PatchPlate in the PatchPlate station. See Patch Engine Sensor Test 2 on page 175.
3. Test with the PatchPlate in the PatchPlate station and with the Model Cell over the PatchPlate on the PatchPlate station. See Patch Engine Sensor Test 3 on page 176.
4. Test with the PatchPlate in the PatchPlate station and with the Electrode-Plate over the PatchPlate on the PatchPlate station. See Patch Engine Sensor Test 4 on page 177.
5. Repeat Patch Engine Sensor Test 1 on page 174 to verify that the capacitance sensors are still responding correctly after completing Patch Engine Sensor Test 1 through Patch Engine Sensor Test 4 activities.

Patch Engine Sensor Test 1

Do Patch Engine Sensor Test 1 with nothing on the process deck.

To do Patch Engine Sensor Test 1:

1. Check the sensor status in **Instrument** > **View Instrument Status** without a PatchPlate, and without an Electrode-Plate or Model Cell on the process deck.

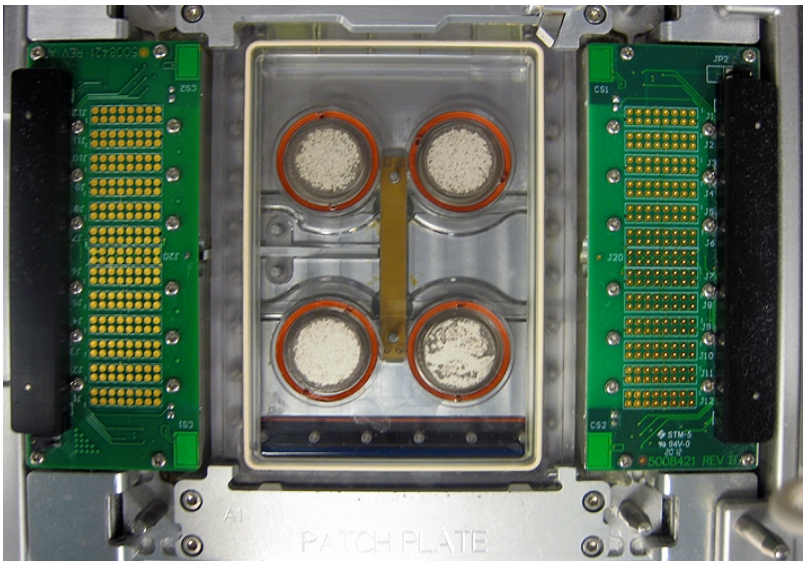


Figure 16-4: Empty PatchPlate Station

2. Click **Update**.
3. Check the sensor status in Instrument > View Instrument Status for PatchPlate and for the Electrode-Plate. See Viewing Instrument Status.

   The **Electrode-Plate Location** status should show **Not Present**, and the **PatchPlate** status should show **Not Present**. See Viewing Instrument Status on page 172.
4. If the **Instrument Status** dialog shows **Electrode-Plate Location**|**Tilted** or **PatchPlate**, clean the capacitance sensors. See Cleaning the Capacitance Sensors on page 179.
5. If the **Instrument Status** dialog shows **Electrode-Plate Location**| **Wash Station**, contact Molecular Devices Technical Support.
6. If the **Instrument Status** dialog shows **PatchPlate**|**Present** or **Tilted**, clean the optical sensors. See Cleaning the Amplifier Optical Sensors on page 181.
7. Continue to the next Patch Engine sensor test. See Patch Engine Sensor Test 2 on page 175.

Note: If you have already cleaned the capacitance sensor, and cleaned the optical sensors, and this test continues to fail, contact Molecular Devices Technical Support.

Patch Engine Sensor Test 2

Do Patch Engine Sensor Test 2 with a PatchPlate on the PatchPlate station and nothing else on the process deck.

To do Patch Engine Sensor Test 2:

1. Place a PatchPlate on the PatchPlate station.

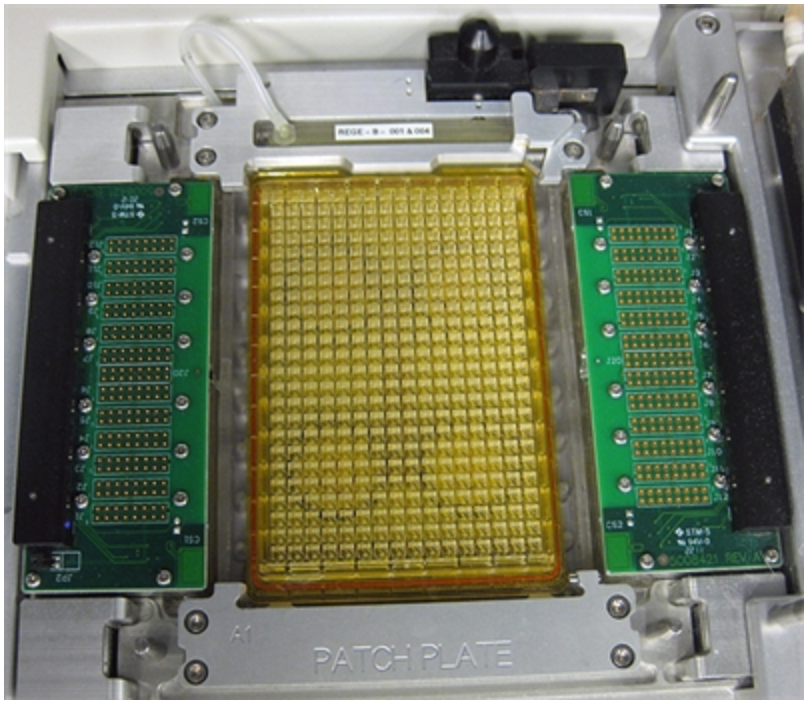


Figure 16-5: PatchPlate on the PatchPlate Station Only

2. Click **Update**.
3. Check the sensor status in **Instrument** > **View Instrument Status** for **PatchPlate** and for the **Electrode-Plate**. See Viewing Instrument Status on page 172.

   The **Electrode-Plate Location** status should show **Not Present**, and the **PatchPlate** status should show **Present**.
4. If the **Instrument Status** dialog shows **PatchPlate| Tilted**, check that the PatchPlate is properly seated in the PatchPlate station, and click **Instrument Status** > **Update**.
5. If the **Instrument Status** dialog shows **PatchPlate| Not Present**, clean the optical sensors. Cleaning the Amplifier Optical Sensors on page 181.
6. Continue to the next Patch Engine sensor test. See Patch Engine Sensor Test 3 on page 176.

Note: If you have already cleaned the optical sensors, and this test continues to fail, contact Molecular Devices Technical Support.

Patch Engine Sensor Test 3

Do Patch Engine Sensor Test 3 with a PatchPlate in the PatchPlate station and with the Model Cell also on the PatchPlate station.

To do Patch Engine Sensor Test 3:

1. Place a PatchPlate on the PatchPlate station, and a Model Cell over the PatchPlate on the PatchPlate station.

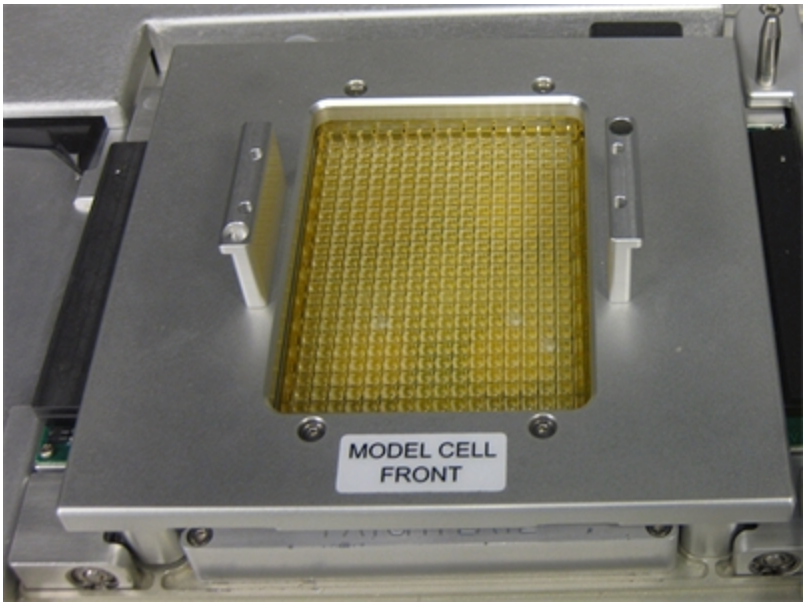


Figure 16-6: Model Cell Clamped Over a PatchPlate on the PatchPlate Station

2. Click **Update**.
3. Check the sensor status in **Instrument** > **View Instrument Status** for the **Electrode-Plate** and **PatchPlate**. See Viewing Instrument Status on page 172.

   The **Electrode-Plate Location** status should show **PatchPlate**, and the **PatchPlate** status should show **Present**.
4. If the **Instrument Status** dialog shows either **Electrode-Plate Location**| **Tilted** or **PatchPlate**| **Tilted**, check that the PatchPlate and Model Cell are properly seated in the PatchPlate station, and click **Instrument Status** > **Update**.
5. If the **Instrument Status** dialog shows any other status for the **Electrode-Plate Location** and **PatchPlate**, clean the capacitance sensors. See Cleaning the Capacitance Sensors on page 179.
6. Continue to the next Patch Engine sensor test. See Patch Engine Sensor Test 4 on page 177.

Note: If you have already cleaned the capacitance sensors, and this test continues to fail, contact Molecular Devices Technical Support.

Patch Engine Sensor Test 4

Conduct Patch Engine Sensor Test 4 with a PatchPlate in the PatchPlate station and with the Electrode-Plate also on the PatchPlate station.

To conduct Patch Engine Sensor Test 4:

1. Place a PatchPlate on the PatchPlate station, and an Electrode-Plate over the PatchPlate on the PatchPlate station.

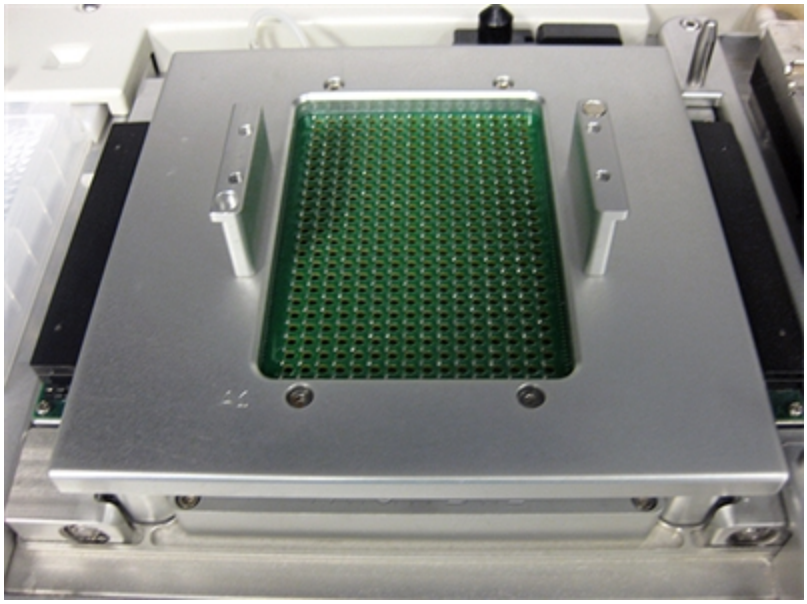


Figure 16-7: Standard Electrode-Plate Clamped Over a PatchPlate on the PatchPlate Station

2. Click **Update**.
3. Check the sensor status in **Instrument** > **View Instrument Status** for the **Electrode-Plate** and **PatchPlate**. See Viewing Instrument Status on page 172.

   The **Electrode-Plate Location** status should show **PatchPlate**, and the **PatchPlate** status should show **Present**.
4. If the **Instrument Status** dialog shows either **Electrode-Plate Location**| **Tilted** or **PatchPlate**| **Tilted**, check that the PatchPlate and Electrode-Plate are properly seated in the PatchPlate station, and click **Instrument Status** > **Update**.
5. If the **Instrument Status** dialog shows any other status for the **Electrode-Plate Location** and **PatchPlate**, clean the capacitance sensors. See Cleaning the Capacitance Sensors on page 179.
6. Continue to the next Patch Engine sensor test. See Patch Engine Sensor Test 5 on page 178.

Note: If you have already cleaned the capacitance sensors, and this test continues to fail, contact Molecular Devices Technical Support.

Patch Engine Sensor Test 5

Do Patch Engine Sensor Test 5 with nothing on the process deck.

To do Patch Engine Sensor Test 5:

1. Repeat Patch Engine Sensor Test 1 on page 174 to verify that the capacitance sensors are still responding correctly after completing Patch Engine Sensor Test 1 through Patch Engine Sensor Test 4 activities.
2. When all of the **Instrument Status** dialog results are correct, then all of the Patch Engine sensors are functioning properly. Continue running your experiments.

Note: If Patch Engine Sensor Test 1 on page 174 continues to fail after you have cleaned the capacitance sensor, cleaned the optical sensors, cleaned the pogo pins, and cleaned the Electrode-Plate and Model Cell, contact Molecular Devices Technical Support.

Testing the Wash Station Sensor

The Wash station sensor test checks that the Wash station sensor is functioning properly. This test requires that the Electrode-Plate be on the Wash station on the process deck, and the **Instrument Status** dialog is open in the software.

To test the Wash station sensor:

1. Place an Electrode-Plate on the Wash station.

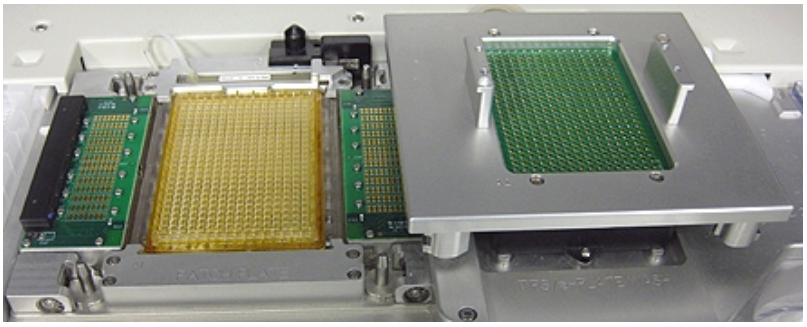


Figure 16-8: PatchPlate on the PatchPlate Station and Electrode-Plate on the Wash Station

2. Click **Instrument Status** > **Update**.

   The **Electrode-Plate Location** status should show **Wash Station**.
3. If the **Instrument Status** dialog shows any other status, contact Molecular Devices Technical Support.

Cleaning the Capacitance Sensors

The capacitance sensors determine if an Electrode-Plate or Model Cell is on the PatchPlate station. The sensors work by measuring the generated capacitance when a metal tab on the underside of an Electrode-Plate or Model Cell is close to the sensing element in the amplifier touch pad boards. The sensors and the metal tabs on the underside of the Electrode-Plate or Model Cell are exposed to liquids and salt solutions contamination.

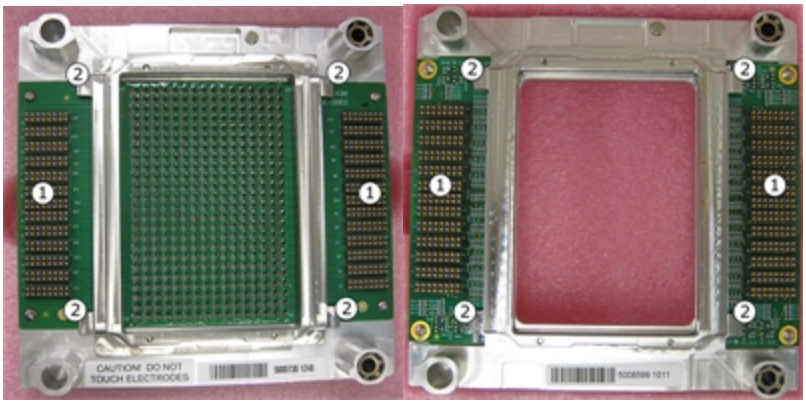


Figure 16-9: Underside of the Standard Electrode-Plate and the Model Cell

Position	Component
1	Pogo pins (connect with amplifier touch pads)
2	Capacitive metal tabs (hover above capacitance sensors)

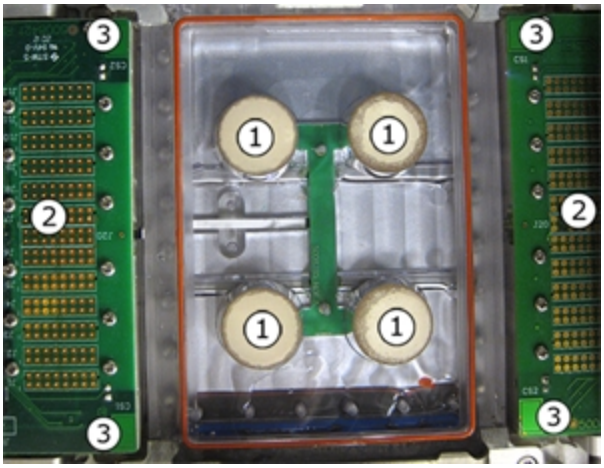


Figure 16-10: The PatchPlate Station Amplifier

Position	Component
1	Standard built-in plenum ground electrodes.
2	Amplifier pogo pin touch pads.
3	Capacitance sensors embedded in the touch pad printed circuit board (PCB)

A contaminated capacitance sensor generally gives erroneously high readings. This capacitance is proportional to the distance between the sensor and the above hovering metal tab. A threshold value, calculated during calibration, determines whether an Electrode-Plate or Model Cell is on the PatchPlate station. A contaminated capacitance sensor can also cause **The Electrode-Plate is tilted** error message to appear in the IonWorks Barracuda® System Software.

When the error messages appear, you may need to clean the capacitance sensors and the capacitive metal tabs on the Electrode-Plate or Model Cell, depending on which you are using at the time of the error message appearance. See Cleaning the Electrode-Plate and Model Cell on page 163.

CAUTION! Always wear gloves when touching parts of the process deck, including an Electrode-Plate or Model Cell. Oils on your hands can damage the equipment and the fluids used for the experiments are hazardous.

To clean the capacitance sensors:

1. Moisten a lint-free cloth with distilled water, and wipe off any salt deposit contaminants that built-up on the capacitance sensors.
2. Moisten a lint-free cloth with isopropyl alcohol, and wipe off any oily contaminants that built-up on the capacitance sensors.

   CAUTION! Do not pour any liquids directly on to the PCBs. Doing so can damage the circuitry.

3. Let the cleaned amplifier capacitance sensors dry completely before continuing.

   CAUTION! Before retesting **Instrument Status**, to avoid damage, make sure all of the printed circuit boards (PCBs) on the touch pads and Electrode-Plate, or Model Cell are completely dry.

4. Verify that the capacitance sensors are clean using the Patch Engine sensor tests. See Testing Patch Engine Sensor Status on page 173.

For capacitance sensor cleaning problems, contact Molecular Devices Technical Support.

Cleaning the Amplifier Optical Sensors

There are two sets of PatchPlate station optical sensors, upper optical sensors and lower optical sensors. Dirty or contaminated upper optical sensors can cause **The PatchPlate is tilted** error message to appear in the IonWorks Barracuda® System Software. Dirty or contaminated lower optical sensors will not cause an error message to appear.

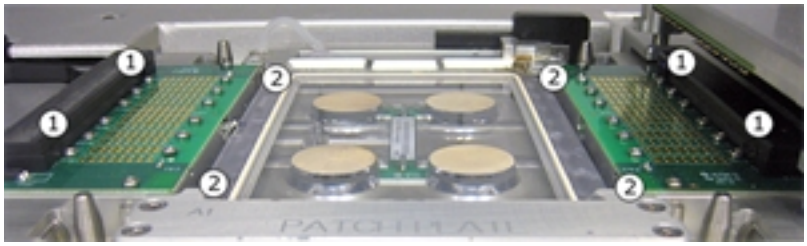


Figure 16-11: The PatchPlate Optical Sensors

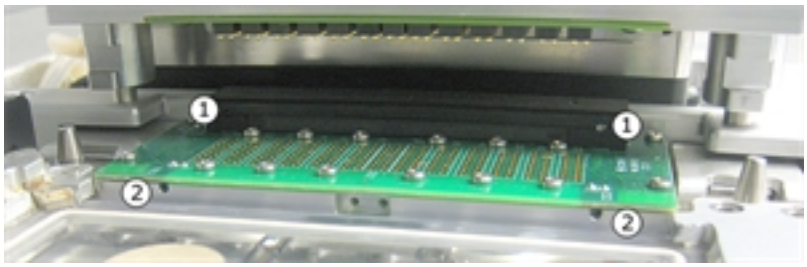


Figure 16-12: The Right Side PatchPlate Optical Sensors

Position	Component
1	Upper optical sensors
2	Lower optical sensors

To clean the optical sensors:

1. Moisten a lint-free cloth or cotton swab with distilled water, and wipe off any salt deposit contaminants that built-up on the lower optical sensors walls.

   CAUTION! Do not press on any sensor. Avoid dislodging the sensors, or causing sensors to become uncalibrated. If a cotton swab is used, make sure no swab cotton is left behind in the sensor holes after wiping. If you dislodge or uncalibrate a sensor, contact Molecular Devices Technical Support.

2. Moisten a lint-free cloth or cotton swab with isopropyl alcohol, and wipe off any oily contaminants that built-up on the lower optical sensors walls.

   CAUTION! Before retesting **Instrument Status**, to avoid damage, make sure all of the printed circuit boards (PCBs) on the touch pads and Electrode-Plate, or Model Cell are completely dry.

3. Verify that the optical sensors are clean using the Patch Engine sensor tests. See Testing Patch Engine Sensor Status on page 173.

For optical sensors cleaning problems, contact Molecular Devices Technical Support.

Calibrating CellPettor

Calibrate CellPettor is a **Utilities** button used for manually running the CellPettor calibration process.

There are situations when the CellPettor calibration sensor prompts you to calibrate the CellPettor. One such situation is clicking **Load CellPettor Pipette > Terminate**.

CAUTION! CAUTION: Always make sure the CellPettor pipette is dry before running calibration. A wet pipette tip can cause inaccurate calibration, or a calibration failure.

To calibrate the CellPettor:

1. In the shortcut bar, click  , and then click **Calibrate CellPettor**.
2. Verify that the CellPettor pipette is dry.
3. Click **Continue**.

   The calibration process is finished when the CellPettor stops in the ready position.

Finding an Amphotericin Pump Tube Leak

You might need to check to see whether the amphotericin peristaltic pump tubing is leaking due to a possible split tube.

To find an amphotericin pump tube leak:

1. Open instrument lower compartment door.

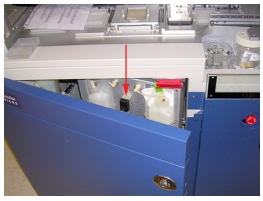


Figure 16-13: The Open Instrument Lower Compartment Door

2. Locate the amphotericin peristaltic pump in the middle of the fluidics-handling system.

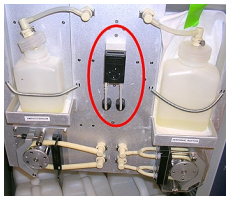


Figure 16-14: The Amphotericin Peristaltic Pump Within the Lower Compartment Fluid-Handling System

3. Open the amphotericin peristaltic pump tubing cover.

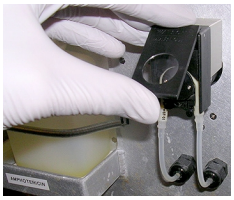


Figure 16-15: The Open Peristaltic Pump Tubing Cover

4. Inspect the tubing.

   If the tubing is worn out and leaking, it looks like Figure 16-16 and needs replacing.

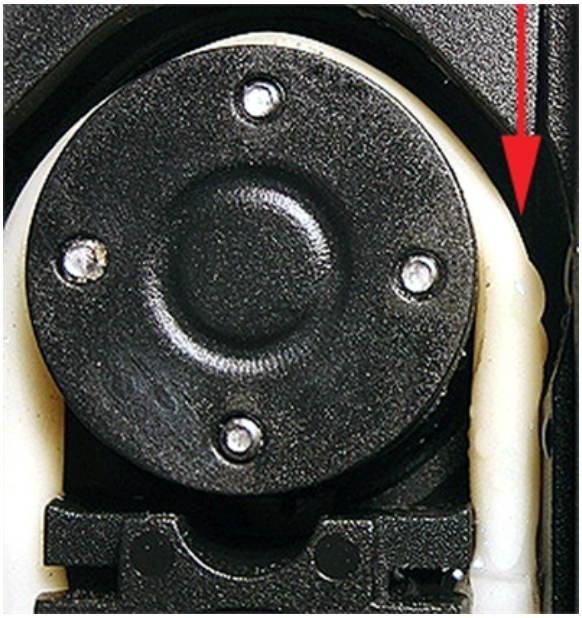


Figure 16-16: Visible Drips of Amphotericin on the Peristaltic Pump Tubing

5. Only replace the amphotericin pump insert tube if you see a visible leak from the tubing. If needed, see Replacing the Amphotericin Pump Insert Tube on page 183.

Replacing the Amphotericin Pump Insert Tube

There is an amphotericin peristaltic pump within the fluid-handling system under the PatchPlate station that is accessible from the lower cabinet of the IonWorks Barracuda System.

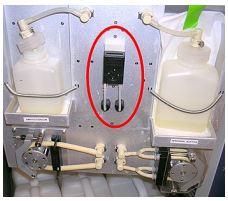


Figure 16-17: The Amphotericin Peristaltic Pump Within the Lower Compartment Fluid-Handling System

To replace the amphotericin pump insert tube:

1. Open instrument lower compartment door.

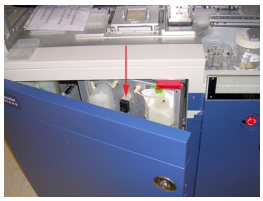


Figure 16-18: The Open Instrument Lower Compartment Door

2. Locate the amphotericin peristaltic pump in the middle of the fluidics-handling system.

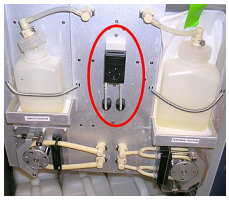


Figure 16-19: The Amphotericin Peristaltic Pump Within the Lower Compartment Fluid-Handling System

3. Open the amphotericin peristaltic pump tubing cover.

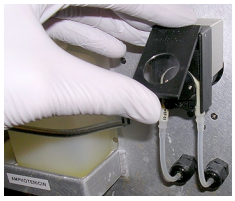


Figure 16-20: The Open Peristaltic Pump Tubing Cover

4. Remove the Tube Insert from the pump by gently stripping it off the rotor wheel.

Figure 16-21: Stripping Tube Insert off of the Rotor Wheel

Figure 16-22: Tube Insert Removed from the Rotor Wheel

5. Disengage the tube insert from the 1/8” outside diameter (OD) silicon tubing.

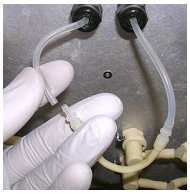


Figure 16-23: Separated Bad Tube Insert from 1/8" OD Tubing

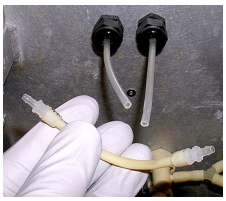


Figure 16-24: Removed Bad Tube Insert

CAUTION! CAUTION: Do not re-install used tubing after it has been removed, even if it is not leaking. Re-installed used tubing fails prematurely because of irregular wearing. Only install new tubing for optimum usage.

6. Attach the replacement tube insert to the 1/8” OD tubing, and verify that there are no gaps.

Figure 16-25: Attaching Replacement Insert Tube

Figure 16-26: Completed Replacement Insert Tube Attachment

7. Loop the replacement tube insert over the rotor wheel and press it into position near the center of the wheel.

Figure 16-27: Looping Replacement Insert Tube Over The Rotor Wheel

Figure 16-28: Pressing Replacement Insert Tube into Position

8. Pull each end of the replacement tube insert in a downward direction to position the clear 7/32” OD tubing sections below the black pump box.

Figure 16-29: Pull Down Replacement Insert Tube to Position Clear 7/32” OD Tubing

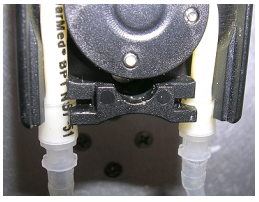


Figure 16-30: Verify Correct Clear 7/32” OD Tubing Position

9. Close the amphotericin peristaltic pump tubing cover.

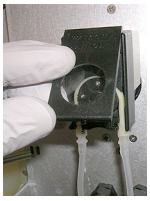


Figure 16-31: Closing Amphotericin Peristaltic Pump Door

Figure 16-32: Amphotericin Peristaltic Pump Tube Replacement Complete

10. Close the instrument lower compartment door.

For amphotericin peristaltic pump tube replacement problems, contact Molecular Devices Technical Support.

Appendix A: List of Approved Fluids

Use only approved fluids with the Automated Patch Clamp System.

CAUTION! Do not use bleach on the surfaces of the instrument, or in the fluid bottles or lines unless specified by a Molecular Devices procedure. Bleach can cause discoloration of bottles, lines, and surfaces, and can cause damage that leads to equipment failure.

Source bottles must be filled prior to the start of an experiment run. This section describes fluids used to fill source bottles, as well as fluids allowed for manual cleaning and rinsing.

Generally, the software controls fluid delivery to the microplates and the wash station from the source bottles below the process deck. You can pour fluids directly into the wash station. Make sure you are using only Molecular Devices approved fluids.

Internal Buffer Bottle

- Buffer solutions
- Deionized (DI) water
- Amphotericin B (Sigma-Aldrich, Cat. A4888)
- Alcohol for cleanup or rinse (Reagent-grade Alcohol Sigma-Aldrich, Cat. R8382) diluted to 50% maximum concentration

Cell Perforation Agent Bottle

- Buffer solutions
- Deionized (DI) water
- Amphotericin B (Sigma-Aldrich, Cat. A4888)
- Dimethyl Sulfoxide (DMSO) solution at 10% maximum concentration
- Alcohol for cleanup or rinse (Reagent-grade Alcohol Sigma-Aldrich, Cat. R8382) diluted to 50% maximum concentration

External Buffer Bottle

- Buffer solutions
- Deionized (DI) water
- Alcohol for cleanup or rinse (Reagent-grade Alcohol Sigma-Aldrich, Cat. R8382) diluted to 50% maximum concentration

CellPettor

- Buffer solutions
- Deionized (DI) water

- Alcohol for cleanup or rinse (Reagent-grade Alcohol Sigma-Aldrich, Cat. R8382) diluted to 50% maximum concentration

Source A and Source B Bottles

- Buffer solutions
- Deionized (DI) water
- Dimethyl sulfoxide (DMSO) solution at 50% maximum concentration
- Alcohol (Reagent-grade Alcohol Sigma-Aldrich, Cat. R8382) diluted to 50% maximum concentration

WARNING! Do not enable ultrasonic cleaning while using any flammable liquid such as alcohol (EtOH) in the Source A or Source B bottles. Sonication can cause flammable liquids to evaporate into the enclosure, posing a potential risk of fire or explosion.

Alcohol Bottle

- Alcohol (Reagent-grade Alcohol Sigma-Aldrich, Cat. R8382) diluted to 50% maximum concentration

Electrode-Plate Rechloriding Boat

- Sodium hypochlorite (Reagent-grade Sodium Hypochlorite Sigma-Aldrich, Cat. 425044) diluted to 50% maximum concentration with 50% DI (deionized) water solution

Suggested Manufacturers and Part Numbers

Follow instructions for maximum concentrations of fluids used in the instrument to avoid damaging the instrument components. The following suggested manufacturers supply suitable fluids:

Source Fluid	Suggested Manufacturer	Catalog Number
Amphotericin B	Sigma-Aldrich	A4888
Reagent-grade Alcohol (EtOH) diluted to 50% maximum concentration	Sigma-Aldrich	R8382
Reagent-grade Sodium Hypochlorite (NaClO) diluted to 50% maximum concentration	Sigma-Aldrich	425044

Appendix B: Accessories

This appendix describes replacement parts and consumable labware used with the IonWorks Barracuda® Automated Patch Clamp System.

Description	Suggested Manufacturer	Part Number
Standard Electrode-Plate Kit (includes Rechloriding Bleach Boat)	Molecular Devices	5012131
RA1 Electrode-Plate Kit (includes Rechloriding Bleach Boat)	Molecular Devices	5027525
Replaceable Ground Electrode (REGE) Kit	Molecular Devices	5031299
Model Cell Kit (includes Rechloriding Bleach Boat)	Molecular Devices	5012133
PatchPlate: Single-Hole (SH)	Molecular Devices	5008679
PatchPlate: Population Patch Clamp (PPC)	Molecular Devices	5008680
External Buffer Boat	Molecular Devices	5029645
Standard Cell Boat	Molecular Devices	5005252
CellPettor Pipette, 5 mL (case of 200)	Molecular Devices	5012605
Conical Tube, 15 mL	BD Falcon	352096
384-Pipettor Tip Disposable Tip Rack (50 racks per case)	Molecular Devices **Note:** Do not use FLIPR system black tips [PN 9000-0764] or clear tips [PN 9000-0763] The IonWorks Barracuda system tips have a reinforced rack.	Black tips 5011976 (recommended) Clear tips 5011975

CellPettor Pipette

The CellPettor is a robotic pipetting unit positioned above the process deck and near the cell boat station. The robot requires a standard 5 mL aspirating pipette with the cotton plug removed from the end, and a standard cell boat [Molecular Devices PN 500525] to operate.

The dimensions for the aspirating pipette should be:

- 8 mm diameter x 350 mm length

Conical Cell Tube

Standard 15 mL conical tubes can be used with the CellPettor subsystem to hold cell suspension.

The dimensions for the cell tube should be:

- 17 mm diameter x 120 mm height

Compound Plates

To ensure proper functioning of the pipettors, the compound plate must match one of the types listed in the IonWorks Barracuda® System Software:

- BD-Falcon 384W Flat Clear PPN
- Corning 384W Round Clear PPN
- Greiner 384W Flat Clear PPN
- Greiner 384W Conical Clear PPN
- Greiner 384W Small Volume Clear PS
- Greiner 384W Flat Clear PS
- Greiner 384W Conical Deep Well Clear PPN
- Nunc 384W Round Natural PPN
- Matrix 384W Reservoir V-Bottom PPN
- Axygen Low Profile Reagent Reservoir PPN
- Seahorse Low Profile Reservoir PPN

Appendix C: Instrument Specifications

C

This section lists electrical requirements, instrument dimensions, and space requirements for the Automated Patch Clamp System.

Operational and Environmental Specifications

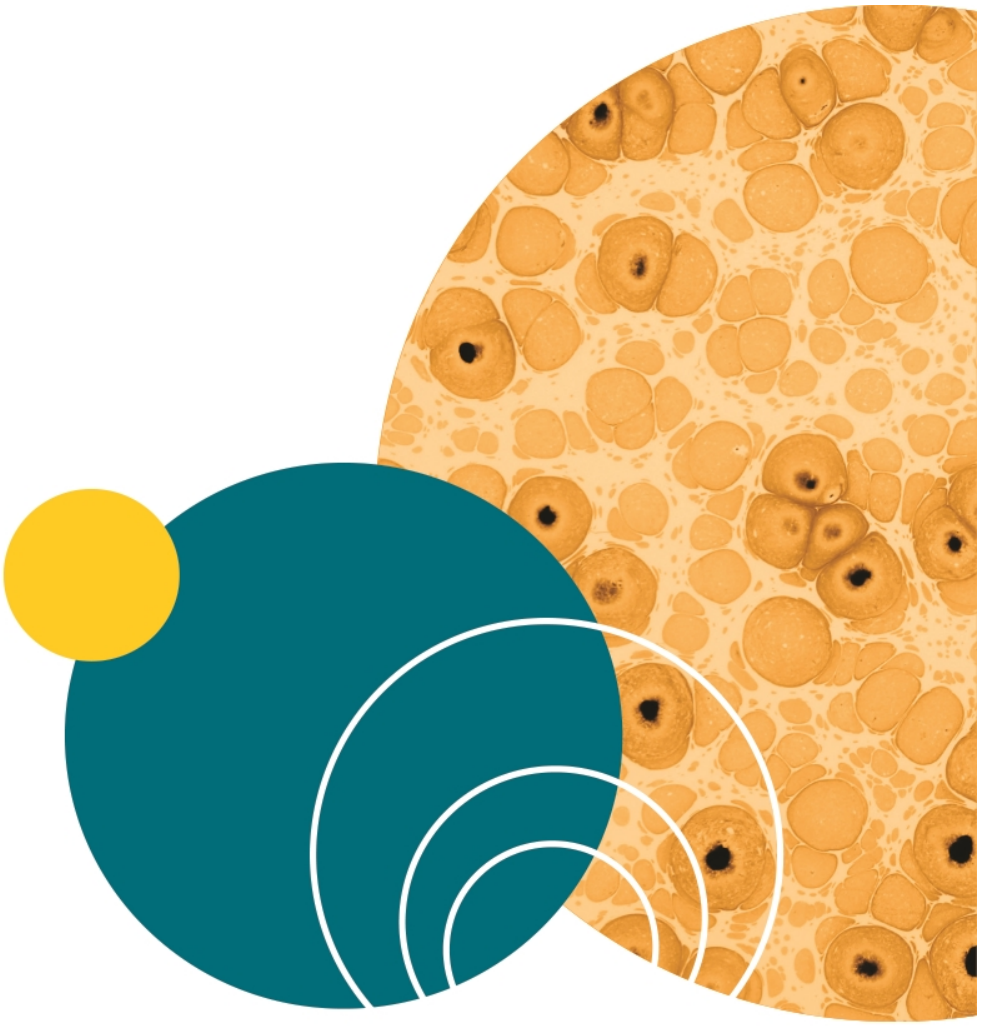
Specification	Measurement
Weight	499 kg [1100 lbs]
Dimensions (H x W x D)	1829 mm high x 1219 mm wide x 838 mm deep [72 inches high x 48 inches wide x 33 inches deep] (with access door closed)
Mains Power Input	100 to 240 VAC, 50/60 Hz, 12 A maximum
Mains Voltage Fluctuations	Not to exceed ±10% of nominal supply voltage
Equipment Class	1
Pollution Degree	2
Installation Category	2
Operating Environment	Indoor Use Only
Altitude	Not to exceed 2000 m
Operating Temperature:	17°C to 27°C
Humidity	35% to 50% non-condensing relative humidity
Ingress Protection:	IP20

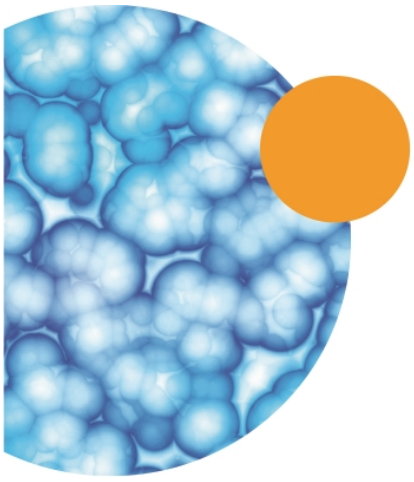
Note: Note: System power supply is configured when manufactured.

Space Requirements

The recommended installation foot print of the IonWorks Barracuda System is 2.334 m wide and 1.1 m deep [92 inches wide and 43 inches deep].

- A minimum clearance of 0.3 m [12 inches] is required to the left side and at the back of the instrument for ventilation and service access.
- A minimum clearance of 0.9 m [36 inches] is required to the right to allow room for the monitor and keyboard bracket.





Appendix D: Safety Guide

This section provides information on the use of user-attention words in this User Guide, precautions to follow before operating the Automated Patch Clamp System, the location of safety labels on the instrument, and a key to understanding safety label icons.

WARNING! If the IonWorks Barracuda System is used in a manner not specified by Molecular Devices, the protection provided by the equipment might be impaired.

WARNING! The IonWorks Barracuda System is an Equipment Class 1 product that relies on protective earth grounding for safe operation. Any interruption of the protective earth ground conductor, inside or outside the instrument, or disconnection of the protective earth ground terminal might result in personal injury.

WARNING! Do not position the equipment so that it is difficult to operate the mains power shut-off switch.

Before Operating the Instrument

Ensure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.
- Read and understood all related Material Safety Data Sheets (MSDS).

Document User-Attention Words

Three user-attention words appear in the text of all Molecular Devices user documentation. Each word implies a particular level of observation or action as described:

Note: Calls attention to useful information.

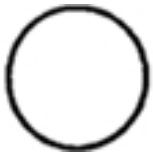
CAUTION! Cautions the user that a potentially hazardous situation could occur, causing injury to the user or damage to the instrument, if this information is ignored.

WARNING! Warns the user that serious physical injury to the user or other persons could result if these precautions are not followed.

Instrument Safety Labels

Safety labels are located on the instrument. Each Safety label consists of a:

- Signal Word panel, which implies a particular level of observation or action (for example, CAUTION or WARNING). If a safety label encompasses multiple hazards, the Signal Word corresponding to the greatest hazard is used.
- Message panel, which explains the hazard and any user action required.
- Safety Alert symbol, which indicates the type of potential personal safety hazard.

Symbol	Indication
	Indicates potential pinch hazard location
	Indicates that product documentation needs to be consulted
	Indicates power on
	Indicates power off
	Indicates the location of the Protective Earth Ground Terminal
	Indicates that you must not discard this electrical/electronic product or its components in domestic household waste

Appendix E: Electromagnetic Compatibility

REGULATORY INFORMATION FOR CANADA (ICES/NMB-001:2006)

This ISM device complies with Canadian ICES-001.

Cet appareil ISM est confomre à la norme NMB-001 du Canada.

ISM EQUIPMENT CLASSIFICATION (Group 1, Class A)

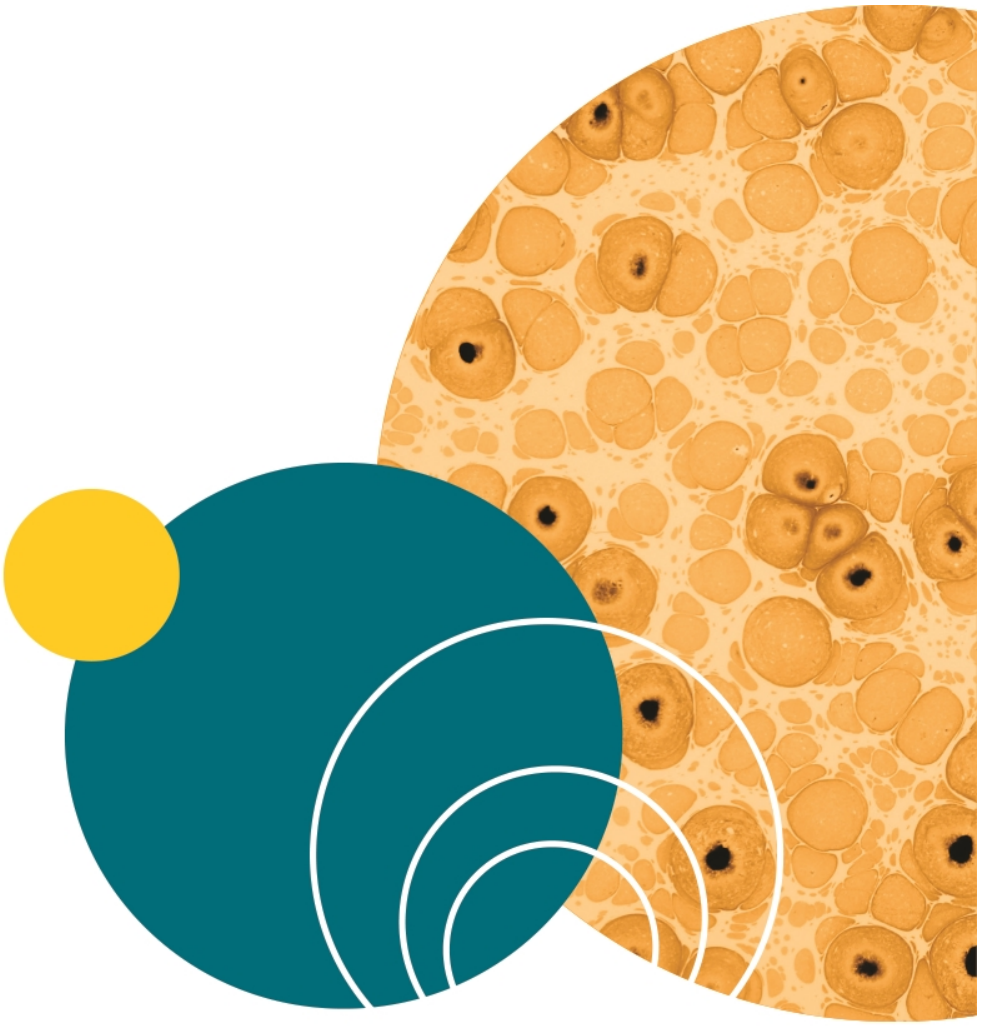
This equipment is designated as scientific equipment for laboratory use that intentionally generate and/or use conductively coupled radio-frequency energy for internal functioning, and are suitable for use in all establishments, other than domestic and those directly connected to a low voltage power supply network which supply buildings used for domestic purposes.

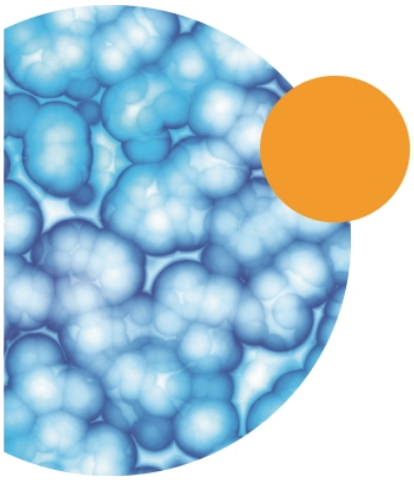
INFORMATION TO THE USER (FCC NOTICE)

This equipment has been tested and found to comply with the limits for non-consumer ISM equipment, pursuant to part 18 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a non-residential installation. This equipment generates, uses, and can radiate radio frequency energy and if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

In order to maintain compliance with FCC regulations, shielded cables must be used with this equipment. Operation with non-approved equipment or unshielded cables is likely to result in interference to radio and TV reception. The user is cautioned that changes and modifications made to the equipment without the approval of the manufacturer could void the user's authority to operate this equipment.





Glossary

Amphotericin B

The recommended cell perforation agent for use in the IonWorks Barracuda™ System (Sigma-Aldrich P/N A4888). General guidelines are 100 µg of cell perforation agent for each milliliter of internal buffer solution.

assay development mode

An experiment mode in which the selected Channel protocol runs and pauses. At the pause, you can adjust parameters. The assay continues to pause after each iteration of the Channel Protocol until you have optimized for your desired result. You can then save the optimized channel protocol as a new protocol. The new optimized channel protocol can then be used in screening mode.

Bleach Boat

A container used to rechloride the electrodes of the Electrode-Plate. The molded surface of the Bleach Boat has a reservoir in the center for bleach and indentations that fit the four Electrode-Plate standoffs. This makes it possible to submerge the electrodes in a bleach solution without submerging the other components of the Electrode-Plate.

buffer boat

An SBS compatible plate with a single trough to hold the external buffer solution. The trough allows dispensing and aspiration of fluids from the 384-well pipettor.

cell boat

An SBS compatible plate with a single trough that contains the cells being used in the assay. The trough allows dispensing and aspiration of fluids from the 384-well pipettor.

CellPettor

An automated cell-handling unit. The CellPettor delivers cell suspension to the cell boat during experiments.

compound plate

An SBS compatible 384-well plate that contains the screening compound. One or more compound plates can be used in the assay.

data aquisition engine

The system that collects signals during assays. Each of the 384 electrodes on the Electrode-Plate connects to a single amplifier and digitizer to simultaneously convert the 384-channel recordings into usable data. This connection is made through the 384 pogo pins on the Electrode-Plate.

electrode array

384 individual electrodes that match the layout of a 384-well plate. The electrode array enables simultaneous compound addition and channel recording.

experiment mode

The mode in which an experiment is run. There are two experiment modes: screening mode and assay development mode.

external buffer

A solution that is dispensed into the PatchPlate by the pipettor. Used as a buffer.

hole test

A test during which the Electrode-Plate records the electrical current after the wells are filled with external buffer, but before cells have been added. A well is defined as "Blocked" when the measured resistance is less than 1 MΩ or greater than 10 MΩ. Wells that do not pass the hole test are excluded when data is analyzed.

internal buffer

A solution that is pumped into the plenum. Used as a buffer.

manual mode

An operating mode in which plates are loaded and unloaded manually.

Model Cell

A component that simulates a whole cell circuit using resistors built into the frame housing. The Model Cell can be used in place of the Electrode-Plate to test basic output and resistance of a voltage application before tesing the protocol on actual cells. Also used to validate new protocols.

offline mode

An operating mode in which the software is not connected to the instrument. Also referred to as simulation mode.

overflow reservoir

A low-capacity overflow sink, or open reservoir, located behind the plenum/cell waste carboy. This reservoir captures any spill or overflow that might occur on the process deck.

PatchPlate

A consumable planar patch microplate that consists of a 384-well, SBS-standard footprint, with a polyimide membrane at the bottom. Also called pPlate. Versions of the PatchPlate include: MegaSeal single hole (SH), and population patch clamp (PPC).

pipette tray

A tray with 384 disposable pipette tips. For each session, the pipettor picks up the pipette tips from this tray and discards them into the tray after the session is complete.

plenum

The fixed foundation of the PatchPlate station. The plenum enables delivery, from below, of internal buffer solution and cell perforation agent solution. The top surface of the plenum contains four ground electrodes.

rechloride

A process that replaces the silver chloride coating on the electrodes of the Electrode-Plate to prevent electrode drift during experiments. The electrodes must be rechlorided when the Standard Deviation of the hole test offset consistently exceeds a threshold amount.

screening mode

An experiment mode in which you select saved or read-only protocols to run without interruption. One Setup Protocol, one Channel Protocol, and one Cleanup Protocol run through to completion except for pre-programmed pauses in operations to add or change compound plates or to add cells.

seal test

A test that determines whether the cell is properly positioned over the hole and sealed in the well. If the cell is not properly sealed as designated by the native filter settings, then the values for that well are excluded from analysis.

system computer

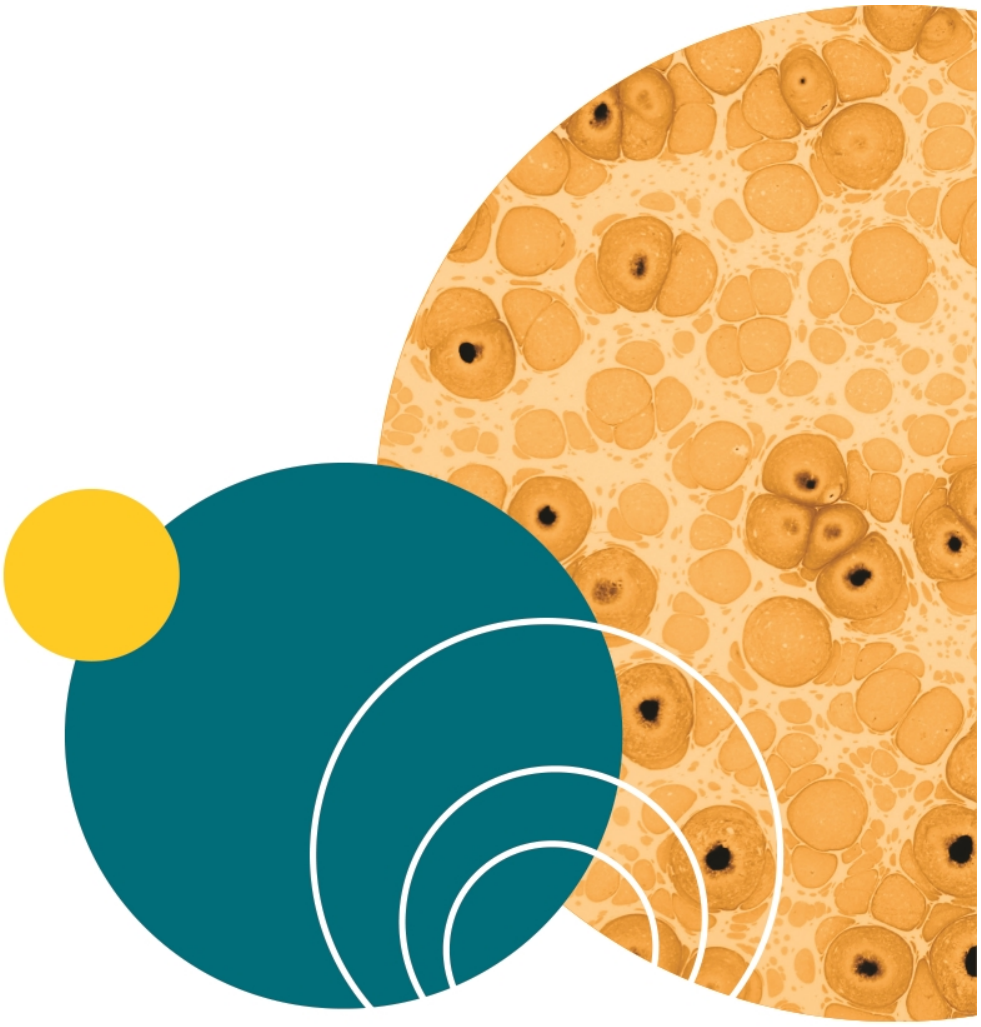
A computer that resides inside the instrument cabinet on the lower-left side. The system computer runs the pre-installed IonWorks Barracuda™ Software that controls automated instrument operations.

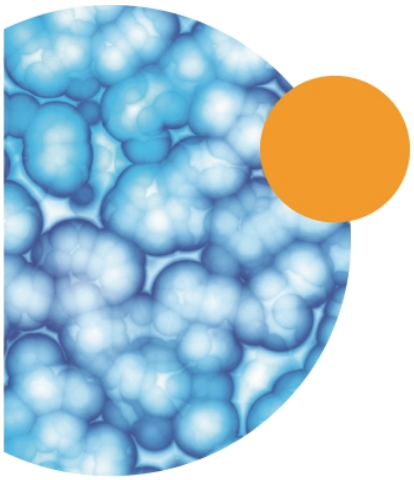
wash solutions

Solutions required to wash the electrodes and pipette tips. They typically consist of DI water, saline solution, 10% DMSO, or 50% Ethanol. The wash solutions are kept in bulk bottles.

waste carboys

Two six-liter waste bottles, housed in the fluidics drawer in the front of the system cabinet (Waste A and Waste B). A third six-liter bottle for (Plenum/Cell Waste) holds cell-contaminated fluids collected from the cell handling system and the plenum including cell suspension, internal buffer, and perforation agent.





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