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IonWorks Barracuda™ Automated Patch Clamp System

Daily Operation Overview

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Molecular Devices

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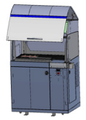
This daily operation overview provides a summary of the basic steps required to use the IonWorks Barracuda™ instrument for a set of experiments. For specific instructions on performing each of these steps, consult the software Help or the User Guide and follow the detailed instructions carefully.



Prepare the Instrument

1. Turn the system on and start the IonWorks Barracuda™ Software.
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, 20 mL of cell perforation agent. Adjust the final volume based on the total number of intended experimental runs. Remember to account for the dead-volume in 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. Make sure a used, undamaged PatchPlate and a new CellPettor pipette are in place. Run the Start of Day Flush and Rinse procedure to eliminate alcohol (EtOH) from the system and prime with saline solutions.
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 patch plate 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.

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.

Select Setup, Channel, and Cleanup Protocols

Choose a Setup Protocol, a Channel Protocol, and a Cleanup Protocol from the list of saved protocols.

- The 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 select the PatchPlate type, run a hole test and a seal test, and obtain cell access before the Channel Protocol starts.
- The 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.
- The Cleanup Protocol performs wash and draining operations after an experiment is complete. The purpose of this protocol is to ensure that all instrument components are clean and ready for the next experiment.
- Edit parameters for each protocol, if applicable. If you are developing a new protocol, select Assay Development Mode to pause the experiment after the Channel protocol runs.

Run the Experiment

- **Screening Mode:** If you are running an experiment in Screening Mode, all three protocols run without interruption. As the data is acquired, review results in the Data Acquisition and Review workspace for the hole test, the seal test, and the progressive results of the voltage-gated or ligand-gated experiment scans.
- **Assay Development Mode:** If you are running an experiment in Assay Development Mode, the instrument pauses after the Channel Protocol. At the pause, edit the Channel Protocol parameters, or load other Channel Protocols, and then run the new protocol. Continue to edit the parameters and run iterations of the Channel Protocol until you have an optimized assay. Rename and save the Channel Protocol to use later in Screening Mode. To complete the experiment, click the option to run the Cleanup Protocol.

Analyze the Data After Acquisition

After the experiment completes and the data is acquired, select the Data Analysis workspace.

- Select the experiment data to review.
- Zoom any well trace to inspect results more closely.
- Edit and apply metrics to reduce data to numeric values.
- Apply filters to remove the less useful data from the results.
- Apply summary criteria to refine hit data.

Export Data

Open the Metric Export dialog to select the scans and User Metrics to include in the exported data. Name the exported data file and set the folder location for data export.

Cleanup

At the end of the day:

1. Remove all labware from the instrument except the PatchPlate and the CellPettor pipette. Provided it is in good condition with no damage, the used PatchPlate can remain in place for the Flush and Rinse procedure.
2. Run the End-of-Day Flush and Rinse procedure to flush and clean the instrument with alcohol (EtOH).
3. Turn the system off.