FLIPR Membrane Potential Assay Kit

The FLIPR® Membrane Potential Assay Kit from Molecular Devices® provides a fast, simple, and reliable fluorescence-based secondary assay for detecting changes in voltage across the cell membrane. This kit is designed to maximize cell line, cell channel, and cell compound applicability while eliminating causes of variability in the data and reducing the number of steps in the conventional protocol.

Table 1-1: Available Kits

Assay Kit	Evaluation Kit	Explorer Kit	Bulk Kit
FLIPR® Membrane Potential Assay Kit	R8128	—	—
FLIPR® Membrane Potential Assay Kit Blue	—	R8042	R8034
FLIPR® Membrane Potential Assay Kit Red	—	R8126	R8123

Product manufactured by Molecular Devices, LLC.
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Molecular Devices, LLC is ISO 9001 registered.

Chapter 1: About FLIPR Membrane Potential Assay Kit

The FLIPR® Membrane Potential Assay Kit from Molecular Devices provides a fast, simple and reliable fluorescence-based secondary assay for detecting changes in voltage across the cell membrane. These kits are designed to work in association with many receptors and ion channels as well as both adherent and non-adherent cell lines. The assay is a mix-and-read procedure in which cells are incubated with the kit reagents for 30 minutes after which the signal is detected using a FLIPR® High-Throughput Cellular Screening System or FlexStation® 3 Multi-Mode Microplate Reader.

There are no intermediate wash steps involved and, typically, the assay is complete one minute after addition of the agonist.

Conventional protocols for evaluating changes in membrane potential are technique-sensitive, multi-step procedures consisting of preparing large batches of dye and introducing dye into the compound plate. Several problems are routinely encountered with the conventional methods, all of which add to experimental variability.

Because ion channel activity is highly sensitive and potentially impacted by subtle chemical changes, two FLIPR® Membrane Potential Assay Kits, Red and Blue, are available to select the optimal conditions for your delicate ion channel targets. Both formulations use Molecular Devices different proprietary quench technology to enhance signal windows and yield acceptable Z-scores to screen a variety of targets, including TRP, ligand-, cyclic nucleotide- and voltage-gated channels. Both formulations, however use the same proprietary indicator dye. Evaluate both assay kits to decide which formulation is best for your target.

Problems in a conventional assay protocol include:

- Slow response time of traditional dyes used
- Extensive pre-read soaking procedure
- Requirement for precise temperature control
- Variation in fluorescence according to ionic concentrations

Molecular Devices has developed the FLIPR Membrane Potential Assay Kits to maximize cell line/channel/compound applicability while eliminating causes of variability in the data and reducing the number of steps in the conventional protocol.

Advantages of the FLIPR Membrane Potential Assay Kits include:

- More reproducible data because washing is not required
- Faster dye response time
- No cumbersome pre-assay preparation
- Ease of use at room temperature to physiological temperatures
- Fewer steps in the assay resulting in higher sample throughput

Chapter 2: Materials and Equipment

Kit Components

FLIPR Membrane Potential Assay Kit

Table 2-1: Components of the FLIPR Membrane Potential Assay Kit

Item	Evaluation Kit (R8128)	Explorer Kit (Blue R8042) (Red R8126)	Bulk Kit (Blue R8034) (Red R8123)
Component A	5 vials Blue 5 vials Red	10 vials	10 vials
Assay Buffer (Component B) 20 mM HEPES buffer + 1X Hank's Balanced Salt solution (HBSS), pH 7.4	1 bottle	1 bottle	—
Assay Buffer (Component B) 200 mM HEPES buffer + 10X Hank's Balanced Salt Solution (HBSS), pH 6	—	—	1 bottle

- The entire Evaluation Kit is sufficient for ten (10) 96-well, 384-well, or fifteen (15) 1536-well microplates. Each vial is sufficient for one (1) 96-well, 384-well, or 1536-well microplate, depending on dead volume of the dispenser used.
- The entire Explorer Kit is sufficient for ten (10) 96-well, 384-well, or fifteen (15) 1536-well microplates. Each vial is sufficient for one (1) 96-well, 384-well, or 1536-well microplate, depending on dead volume of the dispenser used.
- The entire Bulk Kit is sufficient for one-hundred (100) 96-well or 384-well, or one hundred fifty (150) 1536-well microplates. Each vial is sufficient for ten (10) 96-well, or 384-well microplates, or fifteen (15) 1536-well microplates, depending on dead volume of the dispenser used.

Materials Required But Not Provided

Table 2-2: Membrane Potential Assay Consumables and Accessories

Item	Part Number	Suggested Supplier	Phone Number
FLIPR® Membrane Potential Optics Kit: LED Module, 510–545 nm Emission Filter, 565–625 nm	0200-6207	Molecular Devices	+1-800-635-5577 +1-408-747-1700
FLIPR® and FlexStation® validated pipette tips, 96, 384, or 1536 gasket	varies	Molecular Devices	+1-800-635-5577 +1-408-747-1700
Hank's Balanced Salt Solution (10X stock)	14065-056	Thermo Fisher	+1-800-828-6686
HEPES buffer solution 1X	9319	Irvine Scientific	+1-800-437-5706

Storage and Handling

On receipt of the FLIPR Membrane Potential Assay Kit, store Component A contents at room temperature and store Component B contents at 4°C (39.2°F). Under these conditions, the reagents are stable for six (6) months in the original packaging.

After reconstitution, the Loading Buffer is stable for up to eight (8) hours at room temperature. Aliquots can be frozen and stored (without probenecid) for up to one (1) week without loss of activity.

WARNING! Reagents can contain chemicals that are harmful. Exercise care when handling reagents as described in the related safety data sheet (SDS or MSDS). The safety data sheet is available in the Knowledge Base on the Molecular Devices support web site:
https://support.moleculardevices.com/

Compatible Molecular Devices Instruments

The FLIPR Membrane Potential Assay Kit is designed to be used with the following Molecular Devices instruments and their corresponding tips:

- FLIPR Penta High-Throughput Cellular Screening System
- FLIPR® Tetra High-Throughput Cellular Screening System
- FlexStation® 3 Multi-Mode Microplate Reader

Chapter 3: Experimental Protocol

Quick Start Protocol

To run a FLIPR Membrane Potential Assay Kit protocol:

1. Plate cells in microplates and incubate overnight at 37°C (98.6°F), 5% CO_2.
2. Prepare the Loading Buffer the following day.
3. Remove cell plates from the incubator and add an equal volume of loading buffer to each well.
   For example, for a 384-well microplate, add 25 µL of Loading Buffer to 25 µL of cells and media.
4. Return prepared plates to the incubator and incubate 30 minutes at 37°C (98.6°F), 5% CO_2.
5. Prepare compound plates.
6. Run the experiment on a FLIPR instrument or FlexStation 3 instrument.

Cell Handling

The FLIPR Membrane Potential Assay Kit is designed to work with many cell types, both adherent and non-adherent. Standard procedures vary across laboratories and we recognize that a variety of cell handling conditions might be adopted at the discretion of the user. In this section, we provide general guidelines for preparing cells for use with the assay kit.

Adherent cells are the most frequently used cells with the kits. They are typically plated the day prior to an experiment and then incubated in a 5% CO_2, 37°C (98.6°F) incubator overnight. See Table 3-1 for suggested plating volumes and seeding densities to create an 80-90% confluent cell monolayer before placing the plates in a FLIPR instrument or FlexStation 3 reader.

Table 3-1: Suggested Plating Volumes and Seeding Densities

Cell Type (cells/well)	96-well Plate (100 µL growth medium)	384-well Plate (25 µL growth medium)	1536-well Plate (4 µL growth medium)
Adherent cells	20,000–80,000	5,000–20,000	1,500–5,000
Non-adherent cells	40,000–200,000	10,000–50,000	3,000–10,000

For non-adherent cells, we recommend centrifuging cells from culture medium and re-suspending the pellet in culture medium or appropriate buffer of choice on the day of the experiment. Cells can be dye-loaded in a tube or while plated. After the cells are plated, centrifuge the plates at 100 x g for up to 4 minutes with brake off. Alternatively, non-adherent cells can be treated like adherent cells, plating the day before the assay using the same plating volumes and seeding densities, as long as the cells are seeded onto coated plates, such as poly-D-lysine or collagen, to ensure good attachment to the plate bottom.

Preparing Loading Buffer

The following procedure is designed for preparation of the Loading Buffer for either of the FLIPR Membrane Potential Assay Kits, BLUE (R8034) or RED (R8123) in the Bulk format.

Blue or Red

To prepare loading buffer for one Bulk Kit vial:

1. Prepare 100 mL Hanks Balanced Salt Solution (HBSS) + 20 mM HEPES. Adjust to pH 7.4 (Component B) with 1 N NaOH.

Note: Depending upon the cell type and application, the Assay Buffer as provided with the FLIPR Membrane Potential Assay Kit might not be the ideal choice. If so, alternative buffers, such as Tyrode's or PBS, can be used at your discretion to achieve optimal results.

2. Dissolve completely the contents of each Component A vial by adding 100 mL of 1x (96-wells and 384-wells) or 67 mL of 3x (1536-wells) Assay Buffer. Mix by repeated pipetting until the contents are completely dissolved.

Note: For best results, dissolve the contents of each vial with 10 mL of 1x Assay Buffer and vortex for at least 30 seconds. Wash the vial several times using 1x Assay Buffer to yield a total volume of 100 mL (96-wells or 384-wells) or 67 mL (1536-wells).

Note: It is important that the contents are completely dissolved to ensure reproducibility between experiments.

CAUTION! Components supplied are sufficient for proper cell loading. For optimum results, it is important not to add any additional reagents or change volumes and concentrations.

Table 3-2: Required volumes to formulate FLIPR Membrane Potential Assay Kits

Plate Well-Type	Volumes to Formulate FLIPR Membrane Potential Assay Kits	Evaluation Kit (R8128)	Explorer Kit (Red R8126) (Blue R8042)	Bulk Kit (Red R8123) (Blue R8034)
96 or 384	Volume to dissolve Component A (1 vial)	10 mL	10 mL	10 mL
	More required for correct volume	None	None	90 mL
1536	Volume to dissolve Component A	6.5 mL	6.5 mL	10 mL
	More required for correct volume	None	None	55 mL

Loading the Cells Using Loading Buffer

To load the cells:

1. Remove the cell plates from the incubator or centrifuge. Do not remove the supernatant. Add Loading Buffer to each well (100 µL per well for 96-well plates, or 25 µL for 384-well or 2 µL for 1536-well plates).

   Note: Although Molecular Devices does not recommend washing cells before dye loading, growth medium and serum factors can be washed away before adding the Loading Buffer, provided residual volumes after the wash step are as described. Alternatively, cells can be grown in serum-free conditions.

2. Incubate the cell plates for 30 minutes at 37°C (98.6°F).

   Note: In some cases, incubation at room temperature may improve results.

CAUTION! Do not wash the cells after dye loading.

Running the Membrane Potential Assay on FLIPR Instruments

Before incubation, ensure that the FLIPR instrument is equipped with the 510–545 nm LED module and 565–625 nm emission filter. See LED module and emission filter installation instructions in your instrument user guide, or a picture diagram can be sent to you by contacting Molecular Devices Technical Support.

To run a membrane potential assay protocol:

1. Choose the 510–545/565–625 excitation/emission wavelengths respectively from the **Settings** process in ScreenWorks® Software. After incubation, transfer the plates directly to a FLIPR instrument and start the Membrane Potential assay.
2. Place the filter holder in its correct position in the FLIPR instrument.
3. Do the assay signal test before the experiment. The LED intensity, exposure time, and the gate or gain can be adjusted to get the needed RFU range.
   - For a FLIPR Tetra or FLIPR Penta instrument with an EMCCD camera, adjust the typical average baseline counts to a range from 1500 RFU to 2500 RFU.
   - For a FLIPR Penta instrument with an HS EMCCD camera, adjust the typical average baseline counts to a range from 2,500 to 5,000 RFU.
   - For a FLIPR Tetra or FLIPR Penta instrument with an ICCD camera, adjust the typical average baseline counts to a range from 8000 RFU to 10000 RFU.
4. Update the assay signal test to transfer the adjusted settings to the protocol.
5. Before reading the microplate, set up your FLIPR instrument using the recommended experimental setup parameters listed in Recommended Settings for FLIPR Instruments on page 10.

Recommended Settings for FLIPR Instruments

Before reading the microplate, set up your FLIPR instrument using the following recommended protocol settings. Your settings depend on the camera installed in your instrument.

Note: The addition speeds are faster than in the conventional protocol because of the increased robustness of the cells after the new loading procedure. Faster addition speeds can lead to better mixing of compounds and lower signal variance across the plate.

Detection Parameters	EMCCD Camera	HS EMCCD Camera*	ICCD Camera
Read Mode	Fluorescence	Fluorescence	Fluorescence
Camera Mode	—	Normal	—
Excitation Wavelength (nm)	510–545	510–545	510–545
Emission Wavelength (nm)	565–625	565–625	565–625
Excitation Intensity (%)	50[1]	40[1]	50[1]
Camera Gain	50	4	2000
Gate Open	—	—	6[1]%
Exposure Length (s)	0.4[1]	0.02	0.53[1]
Read Interval (s)	1	1	1

* HS EMCCD camera is only compatible with the FLIPR Penta System. Contact Molecular Devices for upgrade information.
1. Can be adjusted to increase or decrease the signal if low RFUs or saturation problems occur.

Addition Parameters	96-Well Plate	384-Well Plate	1536-Well Plate
Compound Addition Volume (µL)	50	12.5	1
Compound Concentration (Fold)	5x	5x	7x
Dispense Speed (µL/s) Adherent Cells	50–100	20–40	2–8
Dispense Speed (µL/s) Nonadherent Cells	10–50	5-20	1-4
Dispense Height	180	30	1

Running the FLIPR Membrane Potential Assay on a FlexStation 3 Instrument

Before reading the microplate, set up the FlexStation 3 instrument using a SoftMax® Pro Software protocol. Use the recommended parameters listed in Table 3-3. Faster transfer rates can lead to better mixing and lower signal variance across the plate.

Molecular Devices recommends that parameters be optimized for each cell line and targeted to deliver the best performance for your assay.

After incubation, transfer the microplates directly to the FlexStation 3 instrument assay plate carriage and run the assay.

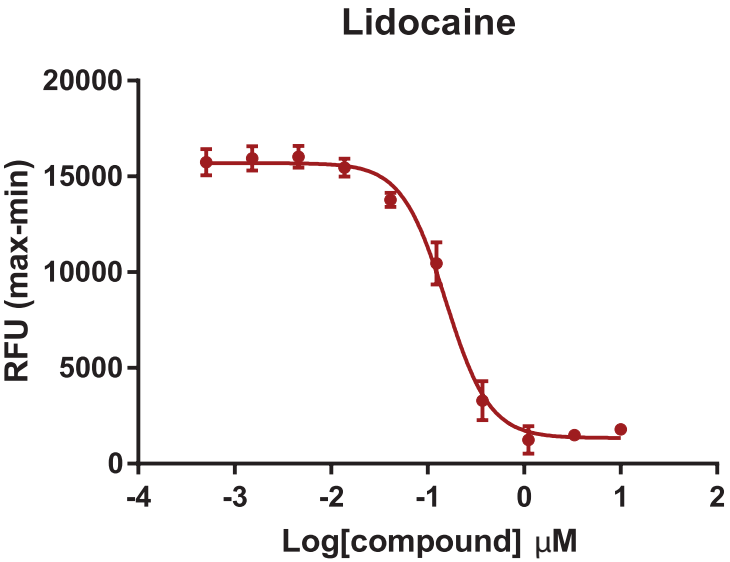
Analyze the data using the SoftMax Pro Software.

Recommended Settings for the FlexStation 3 Instrument

Table 3-3: Experiment Setup Parameters for the FlexStation 3 Instrument

Parameter	96-well microplate	384-well microplate
Excitation Wavelength (nm)	530	530
Emission Wavelength (nm)	565	565
Automatic Emission Cut-Off (nm)	550	550
Pipette Height (µL)	230	40
Transfer Volume (µL)	50	13
Compound Concentration (Fold)	5X	5X
Addition Speed (Rate)	Adherent Cells: 2 to 3 Non-Adherent Cells: 1	Adherent Cells: 5

Data Analysis Examples



Modulation of NaV1.5 channel in CHL cells by lidocaine. Veratridine can be used instead of voltage to open sodium channels as part of a high throughput membrane potential screening strategy to identify modulating compounds. Compared to the IC_{50} value in an electrophysiology assay, the IC_{50} value of lidocaine block in the FLIPR Membrane Potential Assay is 154 nM and is much smaller, which is consistent with previous publications and suggests most NaV1.5 channels in CHL are not at a completely closed state.

Chapter 4: Troubleshooting the FLIPR Membrane Potential Assay Kit

This section presents solutions to problems that users may encounter when running membrane potential assays.

Note: Performance of the Molecular Devices Reagent Kits on Molecular Devices instruments have been validated for use with Molecular Devices pipette tips.

Fluorescence Drop Upon Compound Addition

This may result from dislodging cells from the wells during addition. Shortening incubation times, plating cells on poly-D-lysine plates or slowing the dispense speed should solve the problem in this case.

Adding volumes greater than recommended may increase the initial fluorescence drop. In these cases it may be necessary to adjust the volumes of the components. The recommended volume of the Loading buffer is 100 µL for 96 well plate, 25 µL for 384 well plate, or 2 µL for 1536-well plates.

CAUTION! Increasing the final in-well concentration of the Loading Buffer may decrease the response of the assay. If only one addition is required, then add the appropriate volume of buffer before addition one.

Fluorescence Increase

An increase of fluorescence may be observed upon buffer only challenge. This increase is most likely due to a response of endogenous ion channels to increasing ionic strength. Patch clamping data supports this observed change. The choice of cells and expression levels of endogenous channels can greatly influence resting and changing membrane potentials. Match the compound addition buffer to the buffer in the cell plate (culture medium plus dye loading buffer) so there is no change in ion concentration upon compound addition.

No Response

Not all assays work well with all cell lines or channels. To address this problem, we have developed two different assays to maximize the opportunity for a successful assay. If you have not tried both FLIPR Membrane Potential Assay Kit formulations, Blue and Red, then first try the alternate kit to determine if your cell line is compatible. The FLIPR Membrane Potential Evaluation Assay Kit (R8128) contains both formulations in an Explorer Kit format to facilitate testing of cell lines, channels, and compounds. If you do not see a change in fluorescence with either formulation, then we suggest changing some of the assay conditions.

Recommendations include: replacing media before dye loading, longer incubation and assay times; preparing compounds in the loading buffer; and choosing different buffers such as Tyrode's or specific ion-free buffers. For example, if studying a calcium channel, dye load cells using a calcium-free buffer and prepare your compound plate using a calcium containing buffer.

Effect of DMSO on Membrane Potential Assays

High concentrations of DMSO are toxic to many cell lines and affect compound mixing. Also, DMSO can induce physiological changes in cells (for example, differentiation). If the test compound preparation contains DMSO, it is important to titrate the DMSO to determine the maximum concentration that can be used with each indicator cell line and test compound. With some cell lines that have been tested, there was no effect on signal level up to 1% DMSO final concentration.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website, https://support.moleculardevices.com/, has a link to the Knowledge Base, which contains technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance after consulting the Knowledge Base, you can submit a request to Molecular Devices Technical Support.

You can contact your local representative or Molecular Devices Technical Support at 800-635-5577 (North America only) or +1 408-747-1700. In Europe, call +44 (0) 118 944 8000.

Please have the instrument serial number (on the rear of the instrument), or reagent kit lot number, and any related sample data files available when you call.

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