FLIPR® Membrane Potential Assay Kit (bulk)

Product# R8034 (BLUE)
R8123 (RED)

Introduction

About the Membrane Potential Assay Kit

The FLIPR® Membrane Potential Assay Kits from Molecular Devices Corporation provide a fast, simple and reliable fluorescence-based 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® or FlexStation™ instrument. 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 with FLIPR and FlexStation instruments are technique-sensitive and 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.

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
- Faster response time
- No cumbersome pre-assay preparation
- Ease of use at room to physiological temperatures
- Fewer steps in the assay resulting in higher sample throughput

Applications

The kits provide a homogeneous assay for measuring the membrane potential of a cell population. It is designed to work for the majority of ion channels and receptors that elicit de-polarization of the cell.

Materials and Equipment

Kit Components

The following table lists the kit components.

Table 1: The Membrane Potential Assay Kit (P/N R8034, R8123) contents.

Reagent	Description
R8034 Membrane Potential – BLUE (Bulk Kit)	10 vials Membrane Potential Assay BLUE (Component A), 1 bottle 10X Assay Buffer (Component B) (10X Hanks' BSS with 200 mM HEPES, pH 6) Sufficient for one hundred 96-well or 384-well plates. Each vial is sufficient for assaying ten 96 or 384-well plates.
R8123 Membrane Potential – RED (Bulk Kit)	10 vials Membrane Potential Assay RED (Component A), 1 bottle 10X Assay Buffer (Component B) (10X Hanks' BSS with 200 mM HEPES, pH 6) Sufficient for one hundred 96-well or 384-well plates. Each vial is sufficient for assaying ten 96 or 384-well plates.

Materials Required but not provided

The following tables list the materials required but not supplied.

Table 2: Reagents and supplies

Reagent Item	Source
NaOH and HCl	Sigma® or other general laboratory supplier
FLIPR emission Filter (540-590 nM bandpass)	Molecular Devices PN #0310-4077

Storage and Handling

On receipt of the kit, store the Membrane Potential Assay Kit at room temperature. Under these conditions the reagents are stable for six months in the original packaging.

After formulation, the Loading Buffer is stable for up to eight hours at room temperature. Aliquots can be frozen and stored for up to 5 days without loss of activity.

Membrane Potential Assay Kit Experimental Protocol

Cell Handling

The Membrane Potential Assay Kits are designed to work with many cell types, both adherent and non-adherent. In this section we provide guidelines on how to set up the cells for use with the assay kit.

We recognize that a variety of cell handling conditions might be adopted at the discretion of the user based on standard operating procedures in the laboratory. Optimal cell conditions for the Membrane Potential Assay Kits require the creation of a confluent cell monolayer before placing the plates in FLIPR or FlexStation. In general, we recommend starting with 50,000 - 80,000 cells/well for a 96-well plate and 12,500 - 20,000 cells/well for a 384- well plate.

- For adherent cells, we recommend seeding cells overnight with a plating volume of 100 µl/well for 96-well plates or 25 µl/well for 384-well plates.

- For non-adherent cells, we recommend centrifugation of the cells from culture medium and suspension of the pellet in culture medium. Add 100 µl (96-well plate) or 25 µl (384-well plate) of cell suspension to each well of poly-D-lysine coated plates. It is recommended that you then centrifuge the plates at 1000 rpm for up to 4 minutes **(brake off)**.

1. Preparation of Loading Buffer

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

1.1 To prepare the 1X Assay Buffer, pipette 10 mL of 10x Assay Buffer pH 6.0 (component B) and dilute to 100 mL with distilled water, adjusting to pH 7.4 with NaOH.

Note: Occasionally a white precipitate will form in the 10x Assay Buffer bottle. This is normal and will not affect the assay.

Note: Depending upon the cell type and application, the Assay Buffer as provided with the FLIPR Membrane Potential Assay Kit may not be the ideal choice. If so, alternative buffers may be used at the discretion of the user in order to achieve optimal results.

1.2 Remove one vial of FLIPR Membrane Potential Assay Component A.

1.3 Dissolve contents of vial completely with a final volume of 100 mL of 1x Assay Buffer. Mix by repeated pipetting until the contents are completely dissolved.

Note: For best results, dissolve the contents of the vial with 10 mL of 1x Assay Buffer, then perform several washes of the vial using 1x Assay Buffer to yield a total volume of 100 mL.

Warning: The 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.

2. Loading cells using Loading Buffer

2.1 Remove cell plates from the incubator or centrifuge. Do not remove the supernatant. Add an equal volume of Loading Buffer to each well (100 µl per well for 96-well plates, 25 µl for 384-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.

2.2 Incubate cell plates for 30 min. at 37 °C.

Note: In some cases, incubation at room temperature may work better.

Warning: Do NOT wash the cells after dye loading.

3 Running the Membrane Potential Assay

3.1 FLIPR Assay

3.1.1 Before incubation, remove the filter holder located inside the FLIPR system's filter door. Briefly, release the two thumbscrews holding the filter holder in place and slide the holder out onto a clean or towel-lined bench top. Filter #2 location should be empty. Remove one ring by unscrewing in a counter-clockwise direction. Carefully place the Membrane Potential emission filter in the #2 location and screw the ring back in place with the notches facing outward. Place the filter holder in its correct position in the FLIPR system.

*Note: For more detailed instructions or to have a picture diagram faxed to you, please contact technical services at **800-653-5577**.*

3.1.2 Choose Filter #2 in the experimental setup of the FLIPR software. After incubation transfer the plates directly to FLIPR and begin the Membrane Potential assay. The Membrane Potential assay may be performed at room temperature up to physiological temperature.

3.1.3 Place the filter holder in its correct position in the FLIPR system. For a signal test, a starting average count of 7,000-10,000 RFU is recommended.

3.1.4 Recommended experimental setup parameters are as follows. Note that 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.

Table 1 Experimental setup parameters for 96- and 384-well plates

Parameters	96-well plate	384-well plate
Addition Volume (µl)	50	12.5
Compound Concentration (Fold)	5X	5X
Addition Speed (µl /sec) Adherent cells	50-100	10-20
Addition Speed (µl /sec) Non-Adherent cells	10-20	5-10

3.2 FlexStation Assay

3.2.1 Recommended experimental setup parameters are as follows. Set up your FlexStation using SOFTmax® Pro well ahead of the time you want to read the plate. Faster transfer rates can lead to better mixing and lower signal variance across the plate.

Parameters	
Excitation wavelength (nm)	530
Emission wavelength (nm)	565
Emission cut-off (nm)	550

Parameters	**Addition**
Pipette Height (µL)	230
Transfer Volume (µL)	50
Compound Concentration (Fold)	5X
Addition Speed (Rate) Adherent cells	2-3
Addition Speed (Rate) Non-Adherent cells	1

3.2.2 After incubation, transfer the assay plate directly to the FlexStation assay plate carriage and run the assay.

3.2.3 Analyze the data using SOFTmax Pro.

1. 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 addition speed (RATE) to 10-60 μl/sec (For FLIPR®) or '1' (for FlexStation™) 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 and 25 μl for 384 well plate.

Warning: 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.

2. Increase in fluorescence

You may observe an increase of fluorescence upon buffer only challenge. This increase is most likely due to a response of endogenous ion channels to increasing ionic strength. Patch clamp data supports this observed change. The choice of cells and expression levels of endogenous channels can greatly influence resting and changing membrane potentials.

3. No response

All assays do not 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 Membrane Potential Assay Kit formulations, Blue and Red, then first try the alternate kit to determine if your cell line is compatible. The 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.

Product Use Limitations and Warranty

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