Fluorescence Membrane Potential Assay Kits

Kit Components:

Cat No. mpHTS-Kit OptA: One vial of dye-quencher mixture is provided as a dry solid, or solubilized in DMSO and is suitable for fifty 96-, 384-, or 1536- well plates.

Cat No. mpHTS-Kit OpB. The kit contains six vials with six different combinations of dye-quencher mixtures in DMSO provided as a 500X solution. Each vial is suitable for fifty 96-, 384-, or 1536-wellplates. The kit includes the External Assay Buffer. Volume of dye solution: 1000 μl. Includes 150 ml of 10X External Assay Buffer.

Cat No. MPF-Kit1: This kit contains three vials of dye-quencher mixtures in DMSO (each vial 100 μl volume), with each vial suitable for fluorescence detection of five 96-, 384-, or 1536-well plates. Includes 5 ml of 10X External Assay Buffer.

Cat No. MPF-Kit2: This kit contains three vials of dye-quencher mixtures in DMSO (each vial 200 μl volume), with each vial suitable for fluorescence detection of ten 96-, 384-, or 1536-well plates. Includes 10 ml of 10X External Assay Buffer.

Cat No. MPF-Kit3: This kit contains three vials of dye-quencher mixtures in DMSO (each vial 400 μl volume), with each vial suitable for fluorescence detection of twenty 96-, 384-, or 1536-well plates. Includes 20 ml of 10X External Assay Buffer.

Cat No. MPF-Kit4: This kit contains three vials of dye-quencher mixtures in DMSO (each vial 600 μl volume), with each vial suitable for fluorescence detection of thirty 96-, 384-, or 1536-well plates. Includes 30 ml of 10X External Assay Buffer.

Cat No. MPF-Kit5: This kit contains one vial (100 μl) of dye-quencher mixture in DMSO with each vial suitable for fluorescence detection of ten 96-, 384-, or 1536-well plates. Includes 5 ml of 10X External Assay Buffer.

Cat No. MPF-Kit6: This kit contains one vial (200 μl) of dye-quencher mixture in DMSO with each vial suitable for fluorescence detection of fifteen 96-, 384-, or 1536-well plates. Includes 10 ml of 10X External Assay Buffer.

Cat No. MPF-Kit7: This kit contains one vial (300 μl) of dye-quencher mixture in DMSO with each vial suitable for fluorescence detection of fifteen 96-, 384-, or 1536-well plates. Includes 15 ml of 10X External Assay Buffer

External Assay Buffer (10X): Cat. No. ExtB-2L. Volume; 2L.

Storage and Handling

Upon receipt of the system, store solid powders in the original packaging at room temperature (or -20^{o}C) in the dark for up to 2 years, or for 6 months at -20^{o}C for DMSO suspended dyes.

Warm DMSO suspended dyes to RT prior to use.

A. Cell Handling

Start with ~60,000 cells/well for a 96-well plate and ~15,000 cells/well for a 384-well plate. Extrapolate for other sized culture plates.

- For adherent cells, seed 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, spin down the cells from culture medium and re-suspend 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. Centrifuge the plates at 1000 rpm for up to 4 minutes.

B. Preparation of the Loading Dye

After reconstitution in External Assay Buffer, the loading dye is stable for up to twelve hours at room temperature. Aliquots of dye in External Assay Buffer can be frozen and stored at -20°C for up to two weeks without the loss of activity.

1. Equilibrate the 10x External Assay Buffer to room temperature: Each well of a 96-well dish requires the addition of 100 µl of 2X Loading Dye Solution, whereas each well of a 384-well plate requires 25µl of 2X loading dye solution. Prepare the total volume of 2X Loading Dye Solution according to the number of wells that will be assayed. Dilute the assay buffer in dH_20 to make a 2X Loading Dye Solution at a sufficient volume for your experimental setup. Methods to make 2X lading dye solutions are described below.

   Prior to the assay, wells with cells of a 96-well plate should be submersed in 100 µl of cell culture media without serum (you can also use 1X External Assay Buffer - Cat. No. Cat No. ExtB-2L). Wells of a 384-well plate should be submersed with 25 µl of media without serum (1X External Assay Buffer can be used as well). You should wash out media with serum and replace it with the serum-free cell culture media or the 1X External Assay Buffer in preparation of the assay.

2. Remove one or more vials of dye reagent from the box. For vials with solid-powder, use **Table 1** as a guide to volumes of DMSO required to suspend the dye to make a 500X solution.

   (For vials with the pre-solubilized in DMSO format, the dye solution is pre-configured and shipped as a 500X solution and no further addition of DMSO is needed).

   Table 1. Dye volumes and assay capacity of each kit displayed by the number of 96-, 384- or 1536 well plates per vial.

Part No.	500X Dye Solution Volume of Each Vial («l)	Number of Vials	Number of Plates Per Vial
MPF-Kit1	100	3	5
MPF-Kit2	200	3	10
MPF-Kit3	400	3	20
MPF-Kit4	600	3	30
MPF-Kit5	100	1	5
MPF-Kit6	200	1	10
MPF-Kit7	300	1	15
mpHTS-Kit OptA	1000	1	50
mpHTS-Kit OptB	1000	6	50

3. (For vials with solid- powder: For best results, dissolve the contents of the vial with ½ the final volume of DMSO, then wash the vial using another ½ volume to yield the total volume. If necessary, you may aliquot dye suspended in DMSO at this point prior to storage at -20°C).

4. Suspend the 500X dye solution into the 1X External Assay Buffer to make a 2X Loading dye solution in 1X External Assay Buffer by diluting 500X vials 250 times (e.g. dissolve 100uL of 500X dye in 25mL of 1X Assay Buffer).

5. Use **Table 2** below to make 2X loading dye solutions:

Table 2. Volumes of 1X Assay Buffer to add to 500X dye solutions to make a 2X Loading Dye solution.

Part No.	Dye volume in «l per vial	Dye Concentration Factor	Volume of 1X Assay buffer needed to make 2X Loading Dye solution (mL)
MPF-Kit1	100	500X	25
MPF-Kit2	200	500X	50
MPF-Kit3	400	500X	100
MPF-Kit4	600	500X	150
MPF-Kit5	100	500X	25
MPF-Kit6	200	500X	50
MPF-Kit7	300	500X	75
mpHTS-Kit OptA&B	1000	500X	250

C. Loading Cells Using Loading Dye

1. Remove the cell plates from the incubator or centrifuge. Growth medium and serum factors should be washed away and substituted with either serum-free media or 1X External Assay Buffer. Reiterating from above, 100 µl or 25 µl of serum-free media should be added to a well of a 96-well or 384-well plate, respectively, prior to loading cells with dye.

   Add an equal volume of the 2X Loading Dye in 1X External Assay Buffer to each well (e.g. 100 µl per well for 96-well plates, 25 µl for 384-well plates) to make a final 1X dye solution in the well.

2. Incubate the cell plates for 15-20 minutes in the dark at 37°C to load cells with dye.
   (Cells can also be loaded at room temperature in the dark). Do NOT wash the cells after dye loading.

3. IMPORTANT: Prepare a compound plate that contains dye with maintained loading dye and External Assay Buffer concentrations as when loading cells. This step is critical in order to avoid dye/fluorescence depletion when adding compound into the plate reader

4. Add the compound solutions with the dye and incubate at empirically determined times for your compounds. After incubation, transfer the assay plate directly to the plate reader carriage and run the assay. A washing step is not required for the assay.

Running the Membrane Potential Assay

1. Plate Reader Assay

1.1. Follow the plate reader's manufacturer instructions on setting up optical filters in the range appropriate for membrane potential fluorescence measurements.

For a signal test, a starting average count of 7,000-10,000 RFU is recommended. Recommended filter settings are:

Table 3. Excitation - Emission Characteristics for Detection

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

1.2. Recommended experimental setup parameters for automated compound/agonist additions are as follows. Note that the addition speeds are faster than in conventional protocols. Faster addition speeds can lead to better mixing of compounds and lower signal variance across the plate.

Table 4. Compound addition parameters.

Parameters	**96-well plate**	**384-well plate**
Addition Speed (µl /sec) Adherent cells	50-100	10-20
Addition Speed (µl /sec) Non-Adherent cells	10-20	5-10

Troubleshooting Guide

1. Fluorescence drop upon compound addition:
   This may result from cells that dislodge from the wells. Shortening incubation, pre-coating with poly-D-lysine, or slowing the addition speed should solve the problem in this case.

2. Increase in fluorescence:
   You may observe an increase of fluorescence upon buffer only addition. This increase is most likely due to a response of endogenous ion channels to increasing ionic strength.

3. No response:
   All assays conditions do not necessarily work well with all cell lines or channels. To address this problem, we have developed six different assay formulations to maximize the opportunity for success. Try our alternate dye formulations that are offered to determine if your cell line is compatible with one or more of the formulations.

   If you do not observe a fluorescence change after changing formulations, we suggest changing assay conditions. For example, if studying a calcium channel, load cells with dye in a calcium-free buffer and prepare your compound plate in a calcium-containing buffer.

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Product Use Limitations and Warranty

The membrane potential measurement reagents are for research applications only. Not for diagnostic applications. Reagents may contain chemicals that are harmful. Due care should be exercised to prevent direct human contact with the reagent. FIVEphoton Biochemical products are warranted to meet or exceed the stated specifications. FIVEphoton Biochemicals sole obligation and the customer's sole remedy are limited to replacement of the products free of charge in the event that the product fails to perform as warranted.

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