Screen Quest™ Membrane Potential Assay Kit *Orange Fluorescence*

Introduction

Membrane potential is the difference in voltage between the interior and exterior of a cell. The membrane potential allows a cell to function as a battery, providing power to operate a variety of "molecular devices" embedded in the membrane. In electrically excitable cells such as neurons, membrane potential is used for transmitting signals between different parts of a cell. Opening or closing of ion channels at one point in the membrane produces a local change in the membrane potential, which causes electric current to flow rapidly to other points in the membrane. Ion channels have been identified as important drug discovery targets. The Screen Quest™ Membrane Potential Assay Kit is a homogeneous assay with rapid read time. The kit uses our proprietary long wavelength membrane potential indicator that has enhanced fluorescence upon entering cells. The Screen Quest™ Membrane Potential Assay Kit can detect the membrane potential change due to the opening and closing of the ion channels. It delivers higher throughput than the traditional patch clamp assays.

Kit Components

Components	#35999 (1 plate)	#36000 (10 plates)	#36001 (100 plates)
Component A: MP Sensor	1 vial (15 uL)	1 vial (150 uL)	10 vials (150 uL/vial)
Component B: 10X Assay buffer	1 bottle (1 ml)	1 bottle (10 ml)	1 bottle (100 ml)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	1 bottle (9 ml)	1 bottle (100 ml)	Not included

Assay Protocol (for 1 plate)

Brief Summary

Prepare cells in growth medium → Add MP dye-loading solution (100 µL/well for 96well-plate or 25 µL/well for 384-well-plate) → Incubate at RT for 30 min to 1 hour → Read Fluorescence at Ex/Em=530/570 nm

1. Prepare Cells

1.1 For adherent cells, plate cells overnight in growth medium at 40,000 to 80,000 cells/well/100µl for 96-well or 10,000 to 20,000 cells/well/25µl for 384-well plates.

1.2 For non-adherent cells, centrifuge the cells from the culture medium and then suspend the cell pellets in equal amount of HHBS and MP dye-loading solution (see Steps 2.3 below) at 125,000 to 250,000 cells/well/100µl for 96-well or 30,000 to 60,000 cells/well/25µl for 384-well poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with break off prior to the experiments
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for the membrane potential change.

2. Prepare MP sensor dye-loading solution (for 1 plate)

2.1 Thaw 1 vial of Component A (MP sensor), Component B (10X Assay buffer) and Component C (HHBS) at room temperature before use.
Note1: 15 µl of component A (MP sensor) is enough for 1 plate, un-used Component A can be aliquoted and stored at ≤ -20°C for more than 6 months if the tubes are sealed tightly, avoiding light and repeated freeze-thaw cycles.
Note2: Component B and C can be stored at 4°C for convenience.

2.2 Make 1X assay buffer by mixing 1 mL of Component B (10X Assay buffer) with 9 mL of Component C (HHBS, not included in the kit#36001), mix them well.

Note: 10 ml 1X assay buffer is enough for 1 plate, aliquot and store un-used 1X assay buffer at ≤ -20°C, avoid light and repeated freeze-thaw cycles.

2.3 Make MP dye-loading solution for one cell plate by adding 15 µl of Component A (MP sensor) into 10 ml of 1X assay buffer (from Step 2.2), mixing them well. This working solution is stable for at least 2 hours at room temperature.

3. Run Membrane Potential Assay

3.1 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) MP dye-loading solution into the cell plate.
Note1: If you screen compounds interfere with growth medium and serum factors, then replace the growth medium with equal volume of HHBS buffer before adding the MP dye-loading buffer. Alternatively, cells can be grown in serum-free conditions.
Note2: Do NOT wash the cells after dye loading.

3.2 Incubate the dye-loading plate at cell incubator for 30 minutes.
Note: In some cases, incubation at room temperature for 30 to 60 min may work better.

3.3 Prepare the compound plates by using HHBS or your desired buffer.

3.4 Run the membrane potential assay by monitoring the fluorescence at Ex/Em = 530/570 nm.
Note: It is important to run the signal test before your experiment. Different instruments have their own intensity range. Adjust the signal test intensity to the level of 10% to 15% of the maximum instrument intensity counts. For example, the maximum fluorescence intensity count for FLIPR-384 is 65,000, so the instrument settings should be adjusted to have its signal test intensity around 7,000 to 10,000.

Data Analysis

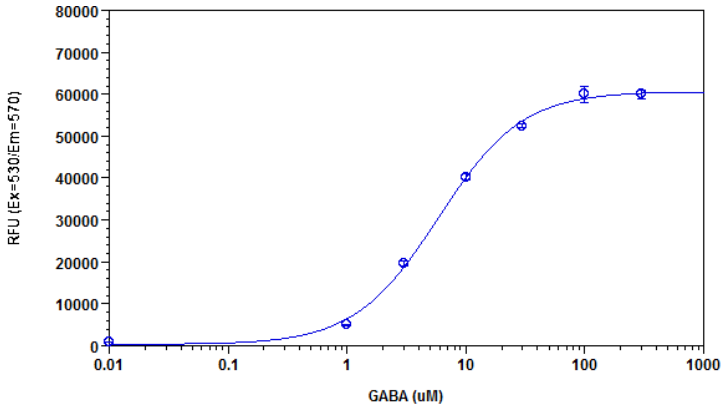

Figure 1. **GABA Dose Response in WSS-1 cells measured with ScreenQuest™ Membrane Potential Assay Kit.** WSS-1 cells were seeded overnight in 50,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µl of the ScreenQuest™ Membrane Potential Assay kit for 30 minutes at room temperature. GABA (50µl/well) was added by FlexStation (Molecular Devices) to achieve the final indicated concentrations.