Screen Quest™ Membrane Potential Assay Kit *Orange Fluorescence*

Catalog number: 35999, 36000, 36001
Unit size: 1 plate, 10 plates, 100 plates

Component	Storage	Amount		
		Cat No. 35999	Cat No. 36000	Cat No. 36001
Component A: MP Sensor	Freeze (<-15 °C), Minimize light exposure	1 vial (15 µL)	1 vial (150 µL)	10 vials (150 µL/vial)
Component B: 10X Assay Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (1 mL)	1 bottle (10 mL)	1 bottle (100 mL)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	Freeze (<-15 °C), Minimize light exposure	1 bottle (9 mL)	1 bottle (100 mL)	Not included

OVERVIEW

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. Our Screen Quest™ Membrane Potential Assay Kit is a homogeneous assay with fast read time. It uses our proprietary long wavelength membrane potential indicator to detect the membrane potential change that is caused by the opening and closing of the ion channels. The red fluorescence of the membrane potential indicator used in the kit has enhanced fluorescence upon entering cells and minimizes the interferences resulted from the screening compounds and/or cellular autofluorescence.

AT A GLANCE

1. Prepare cells in growth medium
2. Add MP dye working solution
3. Incubate for 30 to 60 minutes at room temperature
4. Monitor the fluorescence change before and after the addition of membrane potential altering compounds

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	530 nm
Emission:	570 nm
Cutoff:	550 nm
Instrument specification(s):	Bottom read mode/Programmable liquid handling
Recommended plate:	Black wall/clear bottom

Other Instruments: FLIPR, FDSS, FlexStation, NOVOStar

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Assay Buffer stock solution (1X):
Add 1 mL of 10X Assay Buffer (Component B) into 9 mL of HHBS (Component C, not included in Cat no. 36001) and mix well.

Note 10 mL of 1X Assay Buffer is enough for 1 plate.

PREPARATION OF WORKING SOLUTION

Add 15 uL of MP Sensor (Component A) into 10 mL of 1X Assay Buffer stock solution and mix well. This working solution is stable for at least two hours at room temperature.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) MP dye working solution into the cell plate.

   Note If your screening compounds interfere with growth medium and serum factors, then replace the growth medium with equal volume of HHBS buffer before adding the MP dye working solution. Alternatively, cells can be grown in serum-free conditions.

   Note Do NOT wash the cells after dye loading

2. Incubate the working solution plate in a cell incubator for 30 minutes.

   Note In some cases, incubation at room temperature for 30 to 60 min may work better.

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

4. Run the membrane potential assay by monitoring the fluorescence at Ex/Em = 530/570 nm before and after the addition of compounds using the built in liquid handler in fluorescent microplate reader.

   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.

EXAMPLE DATA ANALYSIS AND FIGURES

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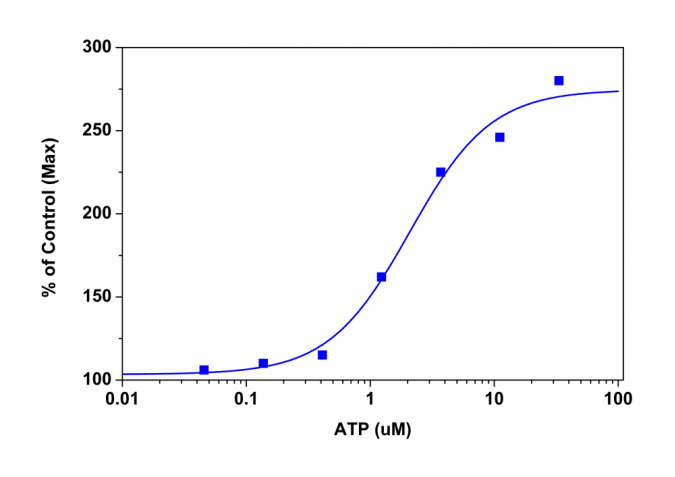

Figure 1.

ATP dose response in HEK cells transiently transfected with P2X receptor. HEK cells transiently transfected with P2X receptor were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of the MP dye-loading solution in a 5% CO2, 37 °C incubator for 60 minutes. ATP (50 µL/well) was added by FlexStation to achieve the final indicated concentrations. The fluorescence signal was measured with bottom read mode at Ex/Em = 530/570 nm (cutoff at 550 nm).