Screen Quest™ Membrane Potential Assay Kit *Red Fluorescence*

Catalog number: 36004, 36005, 36006
Unit size: 1 plate, 10 plates, 100 plates

Component	Storage	Amount		
		Cat No. 36004	Cat No. 36005	Cat No. 36006
Component A: 10X MP Sensor	Freeze (<-15 °C), Minimize light exposure	1 bottle (1 mL)	1 bottle (10 mL/bottle)	10 bottles (10 mL/bottle)
Component B: 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

Protocol summary

1. Prepare cells in growth medium or HHBS
2. Add MP dye-loading solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
3. Incubate at room temperature or 37 °C for 1 hour
4. Monitor the fluorescence intensity at Ex/Em = 620/650 nm

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	620 nm
Emission:	650 nm
Cutoff:	630 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode/Programmable liquid handling

Other Instruments: FDSS, NOVOStar, FlexStation

PREPARATION OF WORKING SOLUTION

MP dye-loading solution:
Add 1 mL of 10X MP Sensor (Component A) into 9 mL of HHBS (Component B), and mix well. Note: The MP dye-loading solution is stable for at least 2 hours at room temperature.

Note 1 mL of 10X MP Sensor is enough for one plate. Unused 10X MP sensor (Component A) can be aliquoted and stored at < -20 °C for a few months, if stored properly. Avoid repeated freeze-thaw cycles.

Note HHBS (Component B) can be stored at 4 °C for convenience.

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) of MP dye-loading solution into the cell plate.

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

   Note DO NOT wash the cells after dye loading.

2. Incubate the dye-loading plate in a 5% CO_2, 37 °C incubator for 30 to 60 minutes.

   Note In some cases, 30 to 60 minutes room temperature incubation may work better.

3. Prepare the compound plates by using HHBS (Component B) or your desired buffer. Prepare the compound plates with HHBS or the desired buffer.

4. Monitor the fluorescence intensity at Ex/Em = 620/650 nm (bottom read).

   Note It is important to run the signal test before the 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.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (% of Control (Max)) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate ATP samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

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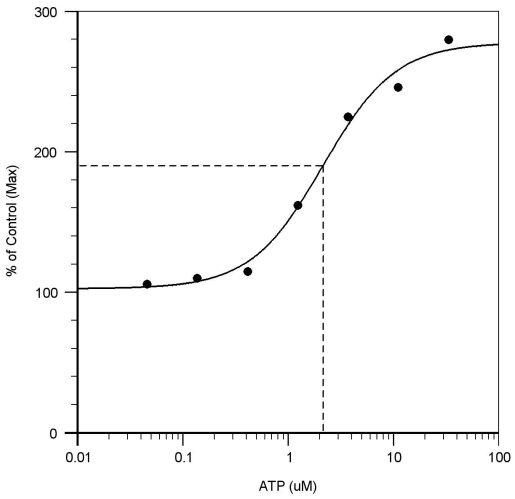

Figure 1. ATP dose response in HEK cells transiently transfected with P2X receptor. HEK cells transiently transfected with P2X receptor were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of the MP dye-loading solution in a 5% CO_2, 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 = 620/650 nm (cutoff at 630 nm).

DISCLAIMER