Screen Quest™ Membrane Potential Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36005 (10 plates), 36006 (100 plates)	Keep in freezer Avoid exposure to light	FLIPR, FDSS, NOVOStar, FlexStation

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.

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.

Kit Components

Components	**Amount**	
	Cat. # 36005 (10 plates)	**Cat. # 36006 (100 plates)**
Component A: 10X MP Sensor	1 bottle (10 mL/bottle)	10 bottles (10 mL/bottle)
Component B: HHBS (Hanks' buffer with 20 mM Hepes)	1 bottle (100 mL)	Not included

Assay Protocol for one 96- well plate

Brief Summary

Prepare cells in growth medium → Add MP dye-loading solution (100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate) → Incubate at RT or 37 °C for 30 minutes to 1 hour → Monitor the fluorescence intensity at Ex/Em = 620/650 nm

1. Prepare Cells:

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

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 Step 2.2 below) at 125,000 to 250,000 cells/well/100 µL for a 96-well poly-D lysine plate or 30,000 to 60,000 cells/well/25 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for the intracellular calcium mobilization.

2. Prepare MP dye-loading solution:

2.1 Thaw one bottle of 10X MP Sensor (Component A), and one bottle of HHBS (Component B) at room temperature before use.
Note1: 1 mL of 10X MP Sensor (Component A) is enough for one plate. Unused 10X MP Sensor (Component A) can be aliquoted and stored at ≤ -20 °C for a few months if the bottle is sealed tightly and kept from light. Avoid repeated freeze-thaw cycles.
Note2: HHBS (Component B) can be stored at 4 °C for convenience.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

2.2 Make MP dye-loading solution for one cell plate by adding 1 mL of 10X MP Sensor (Component A) into 9 mL of HHBS (Compont B), and mixing them well. This MP dye-loading 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) of MP dye-loading solution (from Step 2.2) into the cell plate.
Note 1: 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 2: Do NOT wash the cells after dye loading.

3.2 Incubate the dye-loading plate in a 5% CO_2, 37 ^oC incubator for 30 to 60 minutes.
Note: In some cases, 30 to 60 minutes room temperature incubation may work better.

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

3.4 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.

Data Analysis

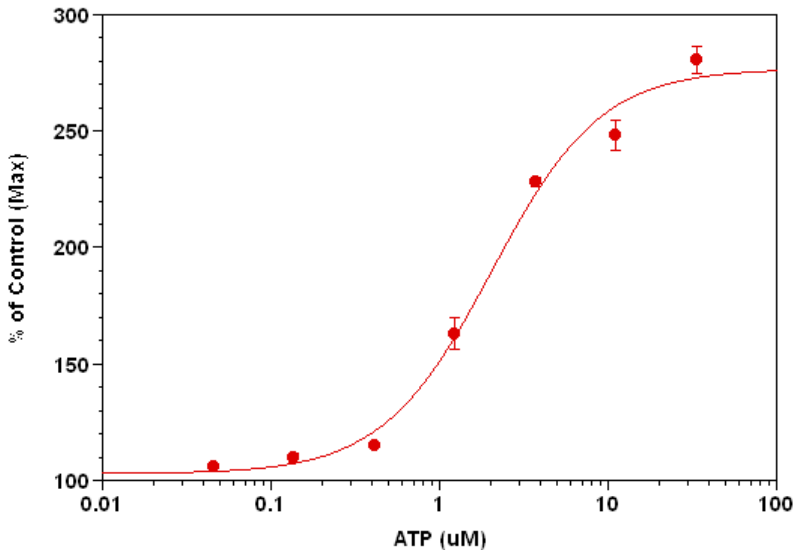

Figure 1. **ATP Dose Response in HEK cells transiently transfected with P2X receptor.** HEK cells transiently transfected with P2X receptor were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of the MP dye-loading solution in a 5% CO_2, 37 ^oC 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).

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.