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Elite™ Potassium Ion Channel Assay Kit

CATALOG NUMBER: CA-P217, (10 plates)

Description

Potassium channels are a diverse and ubiquitous family of membrane proteins present in most cell types and control a wide variety of cell functions. Potassium (K+) channels play key role in regulating such processes as heart rate, hormone secretion, neurotransmitter release, electrolyte and water balance, and cell division/proliferation. Given their physiological importance, K+ channels have emerged as targets of drug discovery efforts for such indications as diabetes, epilepsy, pain, immunomodulation, arrhythmia, dementia, and others.

The Elite™ Potassium Ion Channel kit is a no-wash cell based homogenous assay for high throughput screening measurements of potassium channel activity. Using thallium influx as a surrogate indicator of potassium ion channel activity, the assay is based on the activation of a proprietary fluorescent dye with a high affinity for thallium that reports potassium channel activity with a large fluorogenic signal which is proportional to the number of open potassium channels on the cell. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be read by a fluorescence microplate reader at Ex/Em = 488/525 nm (bottom read).

Kit Components

- **Component A**: Elite™ Thallium Indicator Dye — 1 vial (0.9 mg)
- **Component B**: NYPR Solution, Probenecid Substitute — 1 bottle (5 ml)
- **Component C**: 10x KAB Buffer — 1 bottle (10 ml)

Storage

Keep **Component A** at -20 °C and avoid exposure to light; **Component B** at 4 °C, and **Component C** at room temperature. All components are stable for 6 months after receipt if stored properly.

Materials Required (but not supplied)

- Potassium sulfate (K_2SO_4)
- Thallium(I) sulfate (Tl_2SO_4) (Sigma Cat# 204625-5G)
- Pluronic F-127
- 96 microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader.

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Assay Protocol

1. **Cell preparation:**

1.1 Harvest cells when they reach 80-90% confluence in flasks. Trypsinize cells, and count a portion of the cells with a hemocytometer.

Note: It is very important that the cells DO NOT reach >90% confluence.

1.2 The day before the experiment, plate cells in 384-well, black-walled, clear-bottom plates (e.g. Greiner Bio-One, poly-D-lysine coated) at a concentration of 10 to 20K cells per well.

1.3 Allow cells to attach by leaving the cell plates at room temperature for 30 minutes. Transfer the plates to a cell culture incubator and grow the cells overnight.

2. **Preparation of thallium sulfate in a chloride-free buffer:**

Note: *Thallium is a toxic substance, so it is necessary to use appropriate precautions to prevent inhalation and contact with skin.*

2.1 In a safety hood, carefully open a container of 5 g thallium sulfate (504.8g/mol).

2.2 Add 10 mL of chloride-free buffer, vortex and transfer to a 250 mL graduated cylinder.

2.3 Add an additional 10 mL of chloride-free buffer to the thallium sulfate container, vortex and transfer. Additional washes may be necessary to completely transfer the contents.

2.4 Dilute the thallium sulfate solution to a final volume of 132 mL with chloride-free buffer to give a final concentration of 75 mM Tl_2SO_4; store at 4°C.

2.5 If necessary, vortex or place it into 37°C water bath to effect complete dissolution.

3. **Preparation of dye loading solution:**

3.1. Prepare 1X KAB buffer by adding 90 ml water into 10 ml of 10X KAB buffer.

3.2. Prepare 5 mM Elite™ thallium indicator dye by adding 200 µl DMSO into 0.9 mg of Elite™ thallium indicator dye (**Component A**). Aliquot and store any unused portions at -20°C, protected from light.

3.3. Prepare 20 ml of Dye Loading Solution by adding 16 µl of 5mM Elite™ thallium indicator dye, 400 µl of 5% Pluronic F-127 and 500 µl of 40X NYPR in 19.1 ml of 1X KAB.

4. **Cell loading:**

On the experiment day, remove the medium and replace with 40 µl of Dye Loading Solution. Incubate cells with the dye at room temperature (you can try higher temperatures if experience suggests it) for 30-60 minutes.

5. **Preparation of 5X stimulus buffer**

The composition of this buffer depends on the type of channels to be assayed. Prepare 5X compound solutions by diluting ligands (for ligand gated channels) or K_2SO_4 (for voltage-gated targets) with Tl_2SO_4 in Chloride-free Buffer. Concentrations of Tl_2SO_4 and K_2SO_4 used for assays may need to be optimized for each target channel. Typically, 2.5 mM Tl+ and 10 mM K+ are used as final in-well concentration for a voltage-gated channel, so these would be prepared at 12.5 and 50 mM, respectively in the 5X stimulus buffer.

Weaver and co-workers* describe an assay of a K+/Cl- co-transporter that used a stimulus buffer comprising 125 mM sodium bicarbonate, 12 mM thallium sulfate, 1mM magnesium sulfate, 1.8mM calcium sulfate, 5mM glucose, 10mM Hepes (pH 7.3).

6. **Data analysis:**

When ready to assay, add 10 µl of the test compound into each well with the loaded cells. The final volume is 50 µl (1st addition). Begin reading at standard fluorescein wavelengths (excitation/emission: 488 nm/525 nm) with bottom read mode. Collect data for 5 to 10 minutes as needed.

After finishing 1st reading, take out and the cell plate, incubate at room temperature for another 10-15 minutes (total 20 minutes).

Add 12.5 µl of 5X stimulus buffer into each well with the loaded cells (2nd addition). The final volume is 62.5 µl. Immediately read at standard fluorescein wavelengths (488 nm/525 nm). Collect data for 5 to10 minutes as needed**.**

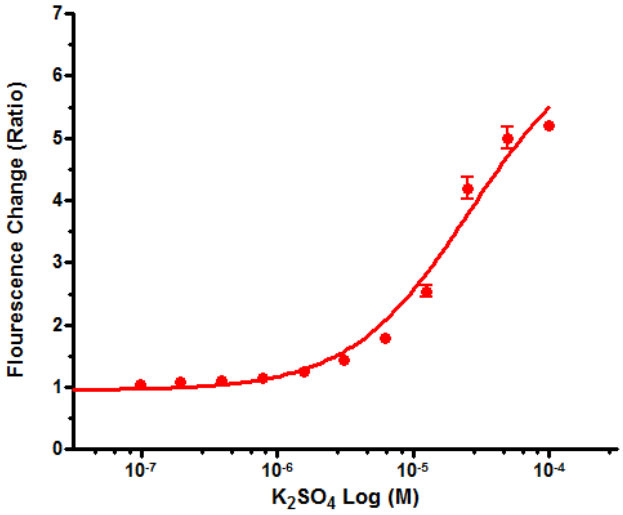

Figure 1. *hERG Channel response to K+ stimulation.* CHO-K1 cells expressing hERG2 were loaded with Elite™ thallium indicator dye for 60 min. The cells were then stimulated with different concentrations of K_2SO_4 in the presence of 2.5 mM Tl_2SO_4. The Data was recorded on Hamamatsu FDSS 7000 instrument.

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