

Technical Data Sheet

Codex KBright™ Potassium Channel Homogeneous Assay Kit

Catalog Number: CB-80500-601 (for 10 plates)

Components

KBright™ thallium indicator dye, 0.9 mg
40X NYPR solution, probenecid substitute, 5 ml
10X KAB buffer, 10 ml

Storage

KBright™ thallium indicator dye be protected from light and stored at -20°C.

40X NYPR solution substitute should be stored at 4°C.

10X KAB buffer should be stored at room temperature

Other materials needed:

Potassium sulfate K_2SO_4

Thallium (I) sulfate Tl_2SO_4 (Aldrich #204625-5G)

Pluronic F-127

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. Prepare Thallium (I) Sulfate in a Chloride-Free Buffer

***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. Prepare Dye Loading Solution

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

3.2 Dissolve 0.9 mg of KBright dye in 200 μ l DMSO and vortex briefly to dissolve the dye completely. This is 5 mM concentration. Aliquot and store any unused portions at -20°C, protected from light.

3.3 Prepare 20 ml of Loading Solution by adding 16 μ l of 5mM KBright, 400 μ l of 5% Pluronic F-127 and 500 μ l of 40X NYPR in 19.1 ml of 1X KAB

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4. Cell Loading

On the experiment day, remove the medium and replace with 40 μ l of 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^- cotransporter 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).

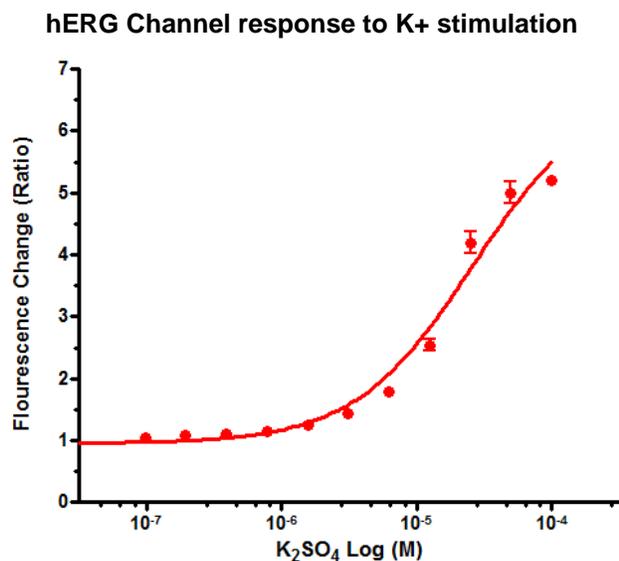
6. Data Recording

When ready to assay, add 10 μ l of the test compound into each well with the loaded cells. The final volume is 50 μ l (1st addition). Begin reading at standard fluorescein wavelengths (488 nm excitation/525 nm emission 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 μ l of 5X stimulus buffer into each well with the loaded cells (2nd addition). The final volume is 62.5 μ l. Immediately read at standard fluorescein wavelengths (488 nm excitation/525 nm emission with bottom read mode). Collect data for 5 to 10 minutes as needed.

Appendix



CHO-K1 cells expressing hERG2 were loaded with KBright™ 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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