ION BIOSCIENCES

Instructions

# Flow Cytometric Potassium Channel Assay

**Table 1 Kit Contents (Cat# 11015-1)**

| Label | Name | Volume | Containers | Storage |
|---|---|---|---|---|
| Reagent A | Thallos AM | 25 µg Vial | 10 | -20° C |
| Reagent B | DMSO[1] | 225 µL Vial | 1 | -20° C |
| Reagent C | 100X Pluronic F-127 | 2 mL Bottle | 1 | 4° C |
| Reagent D | 10X Brilliant Thallium Snapshot Assay Buffer | 10 mL Bottle | 1 | 4° C |
| Reagent E | 50 mM Thallium Sulfate Solution | 4 mL Bottle | 1 | 20—25° C |

## Description

Over the past 15+ years, fluorescence-based measures of $Tl^+$ flux have brought about the discovery of small-molecule modulators of a host of ion channels, transporters, GPCRs and other targets of interest for both drug discovery and basic research.

ION Biosciences' Flow Cytometric Potassium Channel Assay is the first assay solution for single-cell, high-throughput measurements of $Tl^+$ flux through $K^+$, $Na^+$, non-selective cation channels, and some $Na^+$ or $K^+$ transporters. Powered by our patent-pending Snapshot assay technology, ion channel modulation generates a long-lasting signal so you can easily detect and quantify changes in ion channel or transporter activity using Flow Cytometry. Brilliant Thallium Snapshot has effectively identified agonists and/or antagonists of voltage-gated $K^+$ (hERG) and $Na^+$ ($Na_V$) channels, inward rectifying $K^+$ channels (GIRK1/2), and $K^+$ and $Na^+$ transporters ($Na^+/K^+$-ATPase).

ION's Flow Cytometric Potassium Channel Assay provides all the reagents necessary for up to 200 flow cytometry assays. This assay is not compatible with fixed cells.

## Laboratory Procedures

### Getting Started

Before you begin, make sure that you have all the additional reagents and materials you will need for the successful completion of your experiment. While the ION Flow Cytometric Potassium Channel Assay contains all the reagents you will need to prepare your cells for testing, your experiments will likely include other reagents which are not included in your assay package. Notably compounds to be tested are not included, neither are all solvents needed for the dissolution of those compounds. The assay package also does not contain reagents necessary for cell culture or passaging.

In addition to reagents, a flow cytometer with a ~488 nm light source and filter capable of collecting emission at ~520 nm is required (FITC or GFP settings). All flow cytometers and FACS machines on the market can be used with this kit, including BD's Accuri, Beckman Coulter's CytoFlex, MilliporeSigma's Guava EasyCyte, and many others.

## Protocol

These instructions are written for one, microcentrifuge tube containing 1—5 x$10^5$ cells in 0.5 mL. The dye loading solution (Table 2) in this protocol makes enough for 20 independent assays. If fewer assays are needed, adjust volumes accordingly and store the remaining Thallos AM solution at -20ºC for later use. We recommend a maximum of 2 freeze/thaw cycles for best performance. Store all other components at the recommended conditions in Table 1.

1. Add 20 µL DMSO[1] (Reagent B) to the tube containing Thallos AM (Reagent A)
2. Vortex until Reagent A is fully dissolved.
3. Add 8.85 mL of DI water to a 15 mL centrifuge tube.
4. Add 1 mL of 10X Brilliant Thallium Snapshot Assay Buffer (Reagent D) to tube from step 3.
5. Add 100 µL of DySolv (Reagent C) to the tube from step 4.
6. Add 50 µL of Thallium Sulfate solution (Reagent E) to the tube from step 5. While 0.25 mM thallium sulfate works well for many targets, we recommend optimizing this concentration for each assay.
7. Add 20 µL of Thallos AM Solution from step 2 to the tube from step 6.
8. Briefly vortex the tube from step 7 to mix.
9. Prepare cells by centrifuging 1—5 x $10^5$ cells in a microcentrifuge tube. Aspirate culture medium.
10. Resuspend cells in 0.5 mL of dye loading solution (Table 2) from step 8.
11. Keep cells in dark at room temperature for 30 minutes. (Note: Assays can also be performed at 37ºC.)
12. Prepare compound solution(s) and vehicle controls at appropriate concentrations in 1X HEPES-Buffered Hanks Balanced Salt Solution (1X HHBSS, available from ION Biosciences, Table 3), or similar buffer. We recommend a 6X concentration of compound solution(s) when using this protocol. NOTE: For voltage-gated potassium channels, we recommend preparing compounds in a stimulus solution containing 10-20mM $K^+$ to activate the channel. A high-$K^+$, chloride-free stimulus buffer is available from ION Biosciences (Table 3).
13. Add 100 µL of compound solution to cell suspension. Keep cells in dark for an additional 10-30 minutes at room temperature. (Note: While this assay is designed to produce a stable signal for many targets, the kinetics of each assay will need to be optimized for your target, potentiator, and cell type.)
14. Analyze your sample by measuring the fluorescence intensity using FITC settings on your flow cytometer.

**Table 2 Dye Loading Solution**

| Label | Name | Volume |
|---|---|---|
| Reagent A + B | Thallos AM solution | 20 µL |
| Reagent C | 100X Pluronic F-127 | 100 µL |
| Reagent D | 10X Brilliant Thallium Snapshot Assay Buffer | 1 mL |
| Reagent E | 50 mM Thallium Sulfate Solution | 50 µL |
| | Water | 8.85 mL |
| | Total | 10 mL |

## Laboratory Procedures - Footnotes

[1] DMSO is hygroscopic and should be stored tightly closed at -20° C with desiccant pack to prevent the solvent from becoming wet. Wet solvent causes difficulties with dissolution of the dye. Use the DMSO within 6 months of receipt.

## Additional Information

Additional dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department. Custom and bulk sizes are also available. Contact Sales for more information.

| Table 3 | Additional Reagents | Available Sizes | |
|---|---|---|---|
| Kit Label | Name | Size | Catalog # |
| Reagent A | Thallos AM | 500 µg x 1 Vial | 1381C |
| | | 50 µg x 10 Vials | 1381F |
| | | 50 µg x 3 Vials | 1381G |
| Reagent C | 100X Pluronic F-127 | 10 mL Bottle | 7601A |
| Reagent D | 10X Brilliant Thallium Snapshot Assay Buffer | 10 mL Bottle | 7010T-S |
| Reagent E | 50 mM Thallium Sulfate | 5 mL Bottle | 7040S |
| Step 12 | 1X HEPES-Buffered Hanks Balanced Salt Solution (1X HHBSS) | 100 mL Bottle | 7001 |
| Step 12 | 10X High-Potassium ($K^+$), Chloride-Free Stimulus Buffer | 10 mL Bottle | 7030S |