Flow Cytometric Potassium Channel Assay

Table 1	Package Contents			
Label	**Name**	**Volume**	**Containers**	**Storage**
Reagent A	Thallos AM (25 µg)	Dry	10	-20° C
Reagent B	DMSO	225 µL	1	20–25° C
Reagent C	100X Pluronic F-127	2 mL	1	4° C
Reagent D	10X Thallium Snapshot Assay Buffer	10 mL	1	4° C
Reagent E	50 mM Thallium Sulfate Solution	4 mL	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,

Protocol

These instructions are written for one, microcentrifuge tube containing 1—5 x10^5 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 (**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 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 RT for 30 minutes. (Note: Assays can also be performed at 37ºC.)
12. Prepare compound solution(s) and vehicle controls at appropriate concentrations in HHBSS, or similar buffer. We recommend a 6X concentration of compound solution(s) when using this protocol.
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 Thallium Snapshot Assay Buffer	1 mL
Reagent E	50 mM Thallium Sulfate Solution	50 µL
	Water	8.85 mL
	Total	10 mL

Example Results

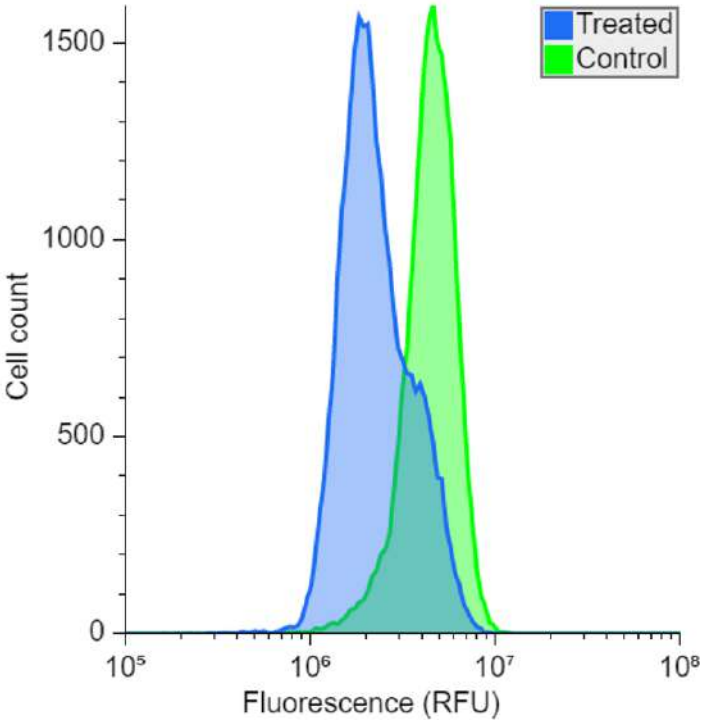

Figure 1. Histogram of CHO GIRK1/2 cell fluorescence. A) Data was acquired 50 minutes after the addition of 1 µM GIRK activator, VU0466551 (VU551) using a BD Accuri C6 Flow Cytometer. Mean cell fluorescence of the treated group is 2.5M RFUs versus 4.5M RFUs for the control, indicating that VU551 is a GIRK channel agonist.

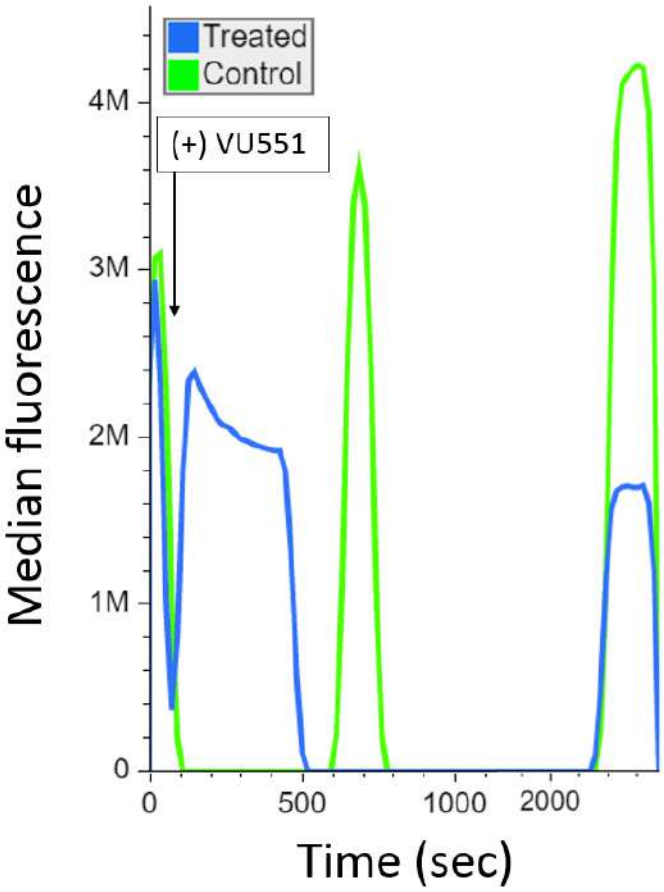

Figure 2. Kinetic profile of CHO GIRK1/2 median cell fluorescence. A) Sample data was acquired before and at different time points after the addition of 3 µM GIRK activator, VU0466551 (VU551) or HHBSS (control). A slow increase in fluorescence is observed in the untreated group. A rapid loss in fluorescence is observed in the treated group, indicating the GIRK channel was activated by VU551.

References

1. Weaver CD, Harden D, Dworetzky SI, Robertson B, Knox RJ. A thallium-sensitive, fluorescence-based assay for detecting and characterizing potassium channel modulators in mammalian cells. J Biomol Screen. 2004 Dec;9(8):671-7.
2. Schmalhofer WA, Swensen AM, Thomas BS, Felix JP, Haedo RJ, Solly K, Kiss L, Kaczorowski GJ, Garcia ML. A pharmacologically validated, high-capacity, functional thallium flux assay for the human Ether-à-go-go related gene potassium channel. Assay Drug Dev Technol. 2010 Dec;8(6):714-26.
3. Delpire E, Days E, Lewis LM, Mi D, Kim K, Lindsley CW, Weaver CD. Small-molecule screen identifies inhibitors of the neuronal K-Cl cotransporter KCC2. Proc Natl Acad Sci U S A. 2009 Mar 31;106(13):5383-8.
4. Niswender CM, Johnson KA, Luo Q, Ayala JE, Kim C, Conn PJ, Weaver CD. A novel assay of Gi/o-linked G protein-coupled receptor coupling to potassium channels provides new insights into the pharmacology of the group III metabotropic glutamate receptors. Mol Pharmacol. 2008 Apr;73(4):1213-24.
5. Carmosino M, Rizzo F, Torretta S, Procino G, Svelto M. High-throughput fluorescent-based NKCC functional assay in adherent epithelial cells. BMC Cell Biol. 2013 Mar 18;14:16.
6. Du Y, Days E, Romaine I, Abney KK, Kaufmann K, Sulikowski G, Stauffer S, Lindsley CW, Weaver CD. Development and validation of a thallium flux-based functional assay for the sodium channel NaV1.7 and its utility for lead discovery and compound profiling. ACS Chem Neurosci. 2015 Jun 17;6(6):871-8.
7. Weaver CD. Thallium Flux Assay for Measuring the Activity of Monovalent Cation Channels and Transporters. Methods

Related Products

Product Code	Product Name
11000-10	Brilliant Thallium Assay, Flex
11011-10	Brilliant Thallium Snapshot Assay
7010TS	10X Brilliant Thallium Snapshot Assay Buffer
11021-10	Brilliant Thallium Gold Snapshot Assay
7040S	50 mM Thallium Sulfate Solution
7300P-50 (50X, 20 mL), 7300P-100 (100X, 20 mL)	Probenecid Solution