ION BIOSCIENCES

Instructions

Brilliant Thallium *Snapshot*

snapshot compatible

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° C *
Reagent C	DySolv	4 mL	1	4° C
Reagent D	10X Thallium Snapshot Assay Buffer	10 mL	1	4° C
Reagent E	TRS	4 mL	1	4° C
Reagent F	Probenecid Solution	4 mL	1	4° C
Reagent G	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' Brilliant Thallium Snapshot Assay is a total assay solution for multi-well plate-based, high-throughput measurements of Tl^+ flux through K^+, Na^+, non-selective cation channels, and some Na^+ or K^+ transporters. Our patent-pending Snapshot assay format generates a long-lasting signal so you can easily detect and quantify changes in ion channel or transporter activity on most fluorescence-capable instruments. So whether you have a FLIPR, a fluorescence microscope, a standard fluorescence plate reader, a high-content imager, or a flow cytometer - you can run functional screens on viable cells using thallium flux - enabling a whole new world of possibilities.

Brilliant Thallium Snapshot simultaneously loads cells with Tl^+ and Thallos AM, ION's Tl^+-sensitive indicator, prior to adding an agonist/antagonist. The addition of an effector compound causes a change in fluorescence, due to Tl^+ efflux or influx, that is sustained for up to 1 hr, depending on your target of interest. 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) using a variety of instruments.

ION's Brilliant Thallium Snapshot assay provides all the reagents necessary for use as a no-wash assay with adherent or non-adherent cells. The optional use of a probenecid solution and an extracellular background masking solution offers the ultimate in compatibility for cell types which are difficult to load with fluorescent Tl^+ indicators (e.g. Chinese Hamster Ovary, CHO cells) and when performing assays in complete, serum-containing cell culture medium is desired.

**Hygroscopic DMSO should be stored at -20° C with desiccant pack to prevent the solvent from becoming wet. Wet solvent causes difficulties with the solubility of the dye. Use the DMSO within 6 months of receipt.*

Description

ION Biosciences Brilliant Thallium Snapshot Assay is also useful for a wide range of effectors of ion channels and transporters including G protein-coupled receptors, lipid kinases and protein kinases. In multi-well, plate-based formats, the Brilliant Thallium Snapshot Assay can be used to discover and characterize the effects of many tens-of-thousands of compounds and environmental factors on effectors of Tl^+ flux.

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 Brilliant Thallium Snapshot Assay package 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.

In addition to reagents, any fluorescence instrument that is capable of providing excitation at ~490 nm and collecting emission at ~520 nm is required. ION's patent-pending Snapshot format generates a sustained signal, which puts an end to the need for a high-speed, parallel plate reader (FLIPR, FDSS, Flexstation, or Panoptic), and makes thallium flux assays accessible on microscopes, high-content imagers, standard fluorescence plate readers, and flow cytometers.

Protocol

These instructions are written for one, 384-well microplate. Certain aspects of the instructions may need to be altered, as appropriate, for multiple microplates or other assay formats (e.g. 96-well microplates or non-adherent cells).

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 appropriate volume of water (Table 2) 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 200 µL of DySolv (Reagent C) to the tube from step 4.
6. Add 200 µL of TRS (Reagent E) to the tube from step 5.
7. If desired, add 200 µL of Probenecid Solution (Reagent F) to the tube from step 6.
8. Add 50 µL of Thallium Sulfate solution (Reagent G) to the tube from step 7. While 0.25 mM thallium sulfate works well for many targets, we recommend optimizing this concentration for each assay.
9. Add 20 µL of Thallos AM Solution from step 2 to the tube from step 8.
10. Briefly vortex the tube from step 9 to mix.

Table 2	Dye Loading Solution	
Label	Name	Volume
Reagent A + B	Thallos AM Solution	20 µL
Reagent C	DySolv	200 µL
Reagent D	10X Thallium Snapshot Assay Buffer	1 mL
Reagent E	TRS*	200 µL
Reagent F	Probenecid Solution**	200 µL
Reagent G	50 mM Thallium Sulfate Solution	50 µL
	Water	8.35 mL
	Total	10 mL

**TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence. Caution is advised when using TRS or other extracellular masking solutions as they may have undesirable effects on assay performance for the target of interest.*

***Probenecid may be included in Dye Loading Solution to aid dye retention. This may be particularly important in certain cell lines (e.g. CHO cells). However, caution is advised when using Probenecid as it may have undesirable effects on assay*

11. Remove the cell-culture medium from the 384-well microplate containing the cells of interest.***
12. Add 20 µL per well of the Dye Loading Solution from step 8 to the microplate from step 9.
13. Incubate the microplate containing the cells and Dye Loading Solution for ~30 minutes at 37C.
14. Prepare compound solution(s) and vehicle controls at appropriate concentrations in HHBSS, or similar buffer. We recommend a 3X concentration of compound solution(s) when using volumes suggested in this protocol.
15. Briefly vortex solutions prepared in step 14 to mix.
16. Add 25 µL per well of solutions from step 14 to an empty 384-well microplate in your desired plate layout.
17. (Optional) Transfer the dye-loaded, cell-containing microplate from step 13 to your instrument of choice. Acquire baseline fluorescence data (F_0) for each well prior to adding compound solution(s) using an excitation wavelength of ~490 nm, and emission wavelength of ~520 nm.
18. Add 10 µL of compound solution(s) prepared in step 16 to the cell-containing plate. Wait 5 - 30 minutes before proceeding to the next step. Wait time will need to be optimized for each assay.
19. Acquire fluorescence data (F) for each well using an excitation wavelength of ~490 nm, and emission wavelength of ~520 nm. Do not change acquisition settings from those used in step 17 if baseline fluorescence (F_0) data was

****Removal of cell culture medium is not required. However, the presence of medium or serum may have negative effects on assay performance. If a no wash assay is preferred, we recommend doubling the volume of Reagents C, E, F, and G in your assay buffer, then adding an equal volume of assay buffer to media in your cell-containing microplate.*

Example Results

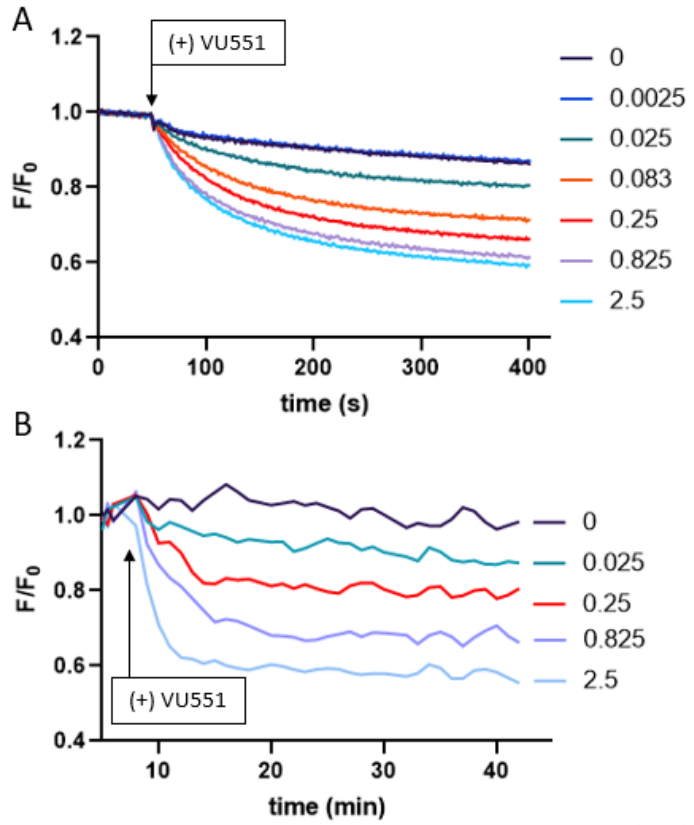
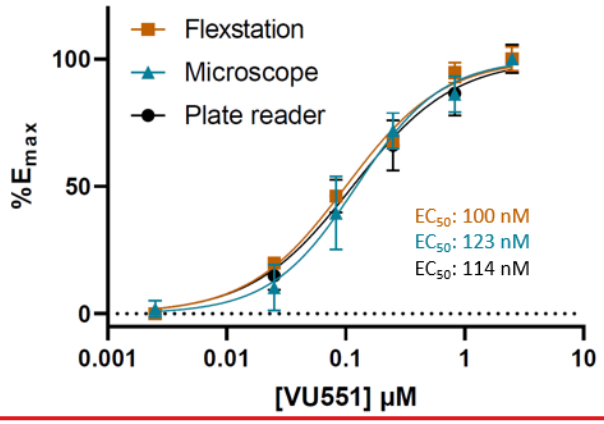


Figure 1. Kinetic fluorescence profiles of CHO GIRK1/2 (G12) cells using Brilliant Thallium Snapshot. A) Cells are pre-loaded with Thallos AM and Tl^+ prior to adding the GIRK activator, VU0466551 (VU551). Kinetic data acquired using a Flexstation. Efflux of Tl^+ upon GIRK channel activation yields a decrease in fluorescence. B) Changes in signal are sustained for >40 minutes as demonstrated using a standard fluorescence plate reader (Cytation 5). Reads were acquired using 1 min intervals. Reported VU551 concentrations are in µM.



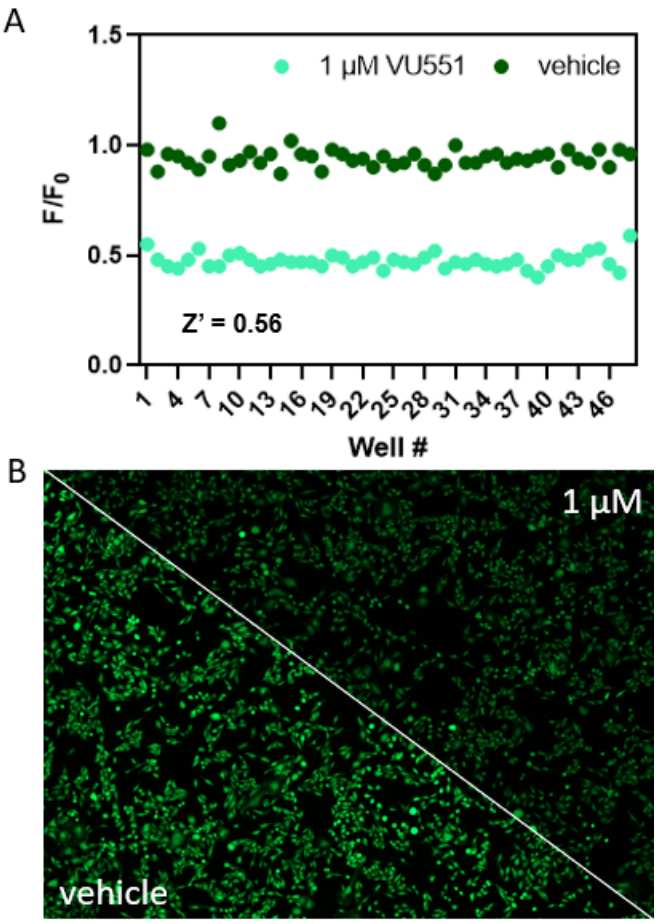


Figure 2. Brilliant Thallium Snapshot assays on a plate reader and microscope. A) Checkerboard layout of a 96-well plate was used to calculate a Z'. Vehicle and activator (1 µM VU551) treated wells are easily identifiable 20 minutes after compound addition (Z' = 0.56). B) Representative fluorescence images of vehicle- (left) and VU551-treated (right) CHO G12 cells demonstrating a 43% reduction in mean cellular fluorescence. Images acquired 20 min after VU551 addition using fluorescein filters and a 4X objective.

Figure 3. Dose-response curves of CHO G12 cells to VU551 measured using many instruments. When using Brilliant Thallium Snapshot Assay, comparable EC_{50} values are obtained on a variety of fluorescence-capable instruments. All values are similar to data acquired using ION's Brilliant Thallium Flex kit (EC_{50} = 77 nM) and published literature (EC_{50} = 75 nM).

Example Results

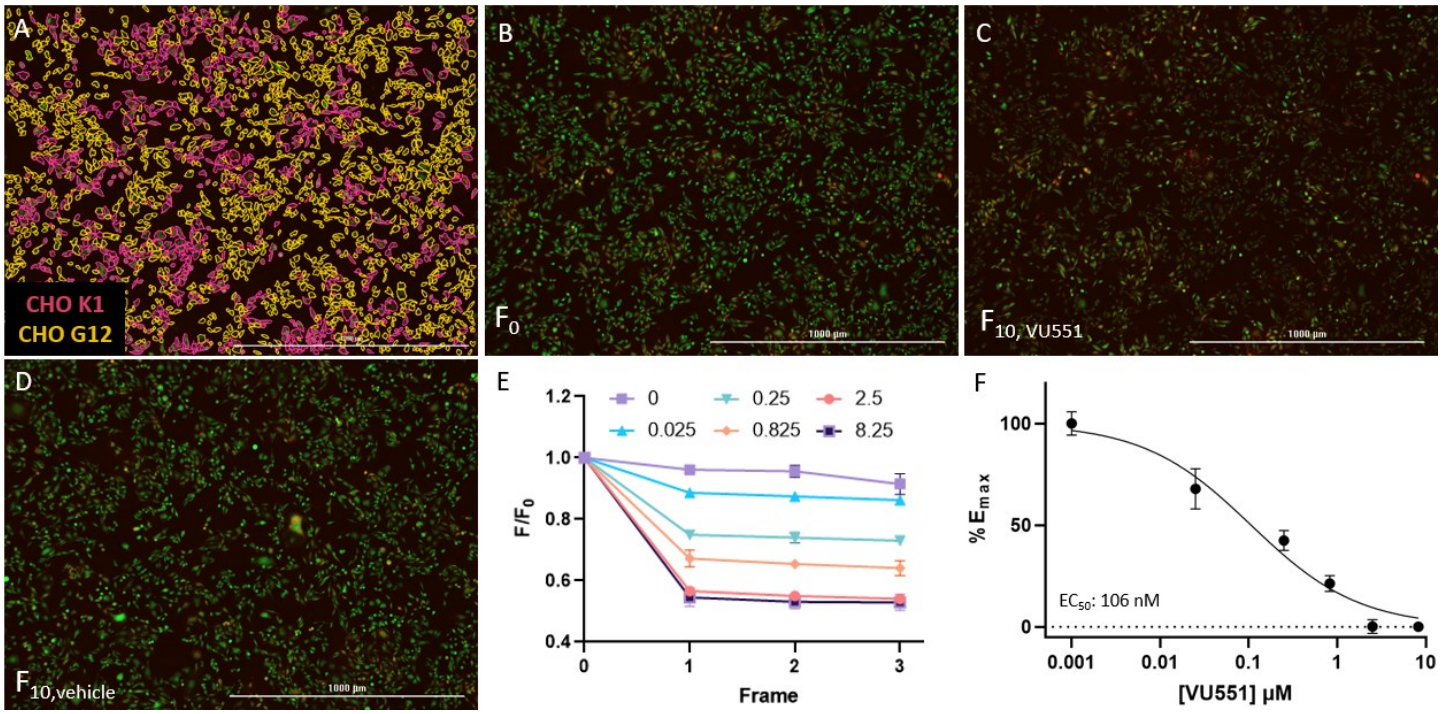


Figure 4. Imaging-based GIRK channel activity data in a co-culture system. A) CHO K1 (pink) cells were stained with CytoTracker Red prior to plating in the same wells as CHO cells overexpressing GIRK1/2 (CHO G12 - yellow). Cytotracker red fluorescence was used to identify cell type. B) Overlay of GFP and TRITC acquired before the addition of VU551 (F_0). C) Overlay of GFP and TRITC images acquired 10 min after the addition of 8.25 µM VU551 (F_{10}). A significant decrease in fluorescence was only observed in CHO G12 cells in the presence of VU551. Images (A - C) display the same field of view. D) Overlay of GFP and TRITC images acquired 10 min after the addition of a vehicle control. No difference in fluorescence between cell types is observed (separate well). E) Change in mean cell fluorescence of CHO G12 cells alone in response to various concentrations of VU551. Frames were acquired every 10 min. Reported VU551 concentrations are in µM. F) Corresponding dose response curve generated using data from Frame 2 - acquired 20 minutes after the addition of VU551.

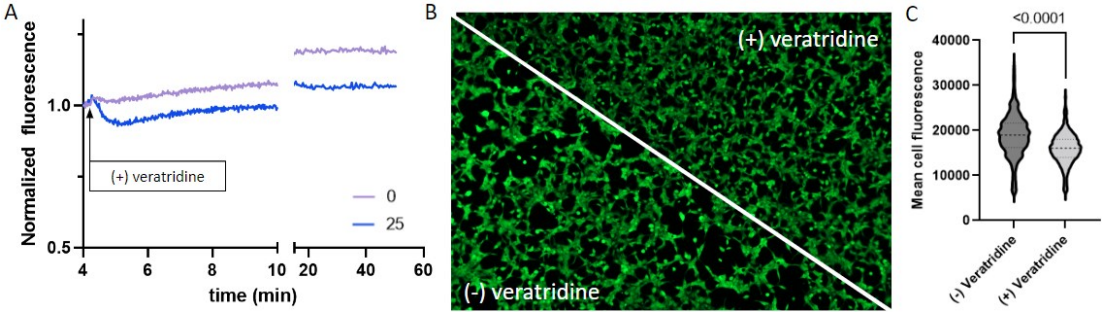


Figure 5. Measuring $Na_V1.3$ activation in engineered HEK293 cells. A) Brilliant Thallium Snapshot Assay reveals a sustained difference in fluorescence between vehicle- and veratridine-activated wells. In this example, a Tl^+ solution was added after dye loading. After 5 min, veratridine (25 µM) was added. Fluorescence was monitored for a total of 45 min after the addition of veratridine. B) Representative fluorescence images of cells in the absence (left) or presence (right) of veratridine. Images were acquired 45 minutes after compound addition using GFP filters. C) Violin plot showing distribution of cell fluorescence in each group. Veratridine-treated cells are significantly less fluorescent than control treated wells based on a Welch's t-test analysis.

Example Results

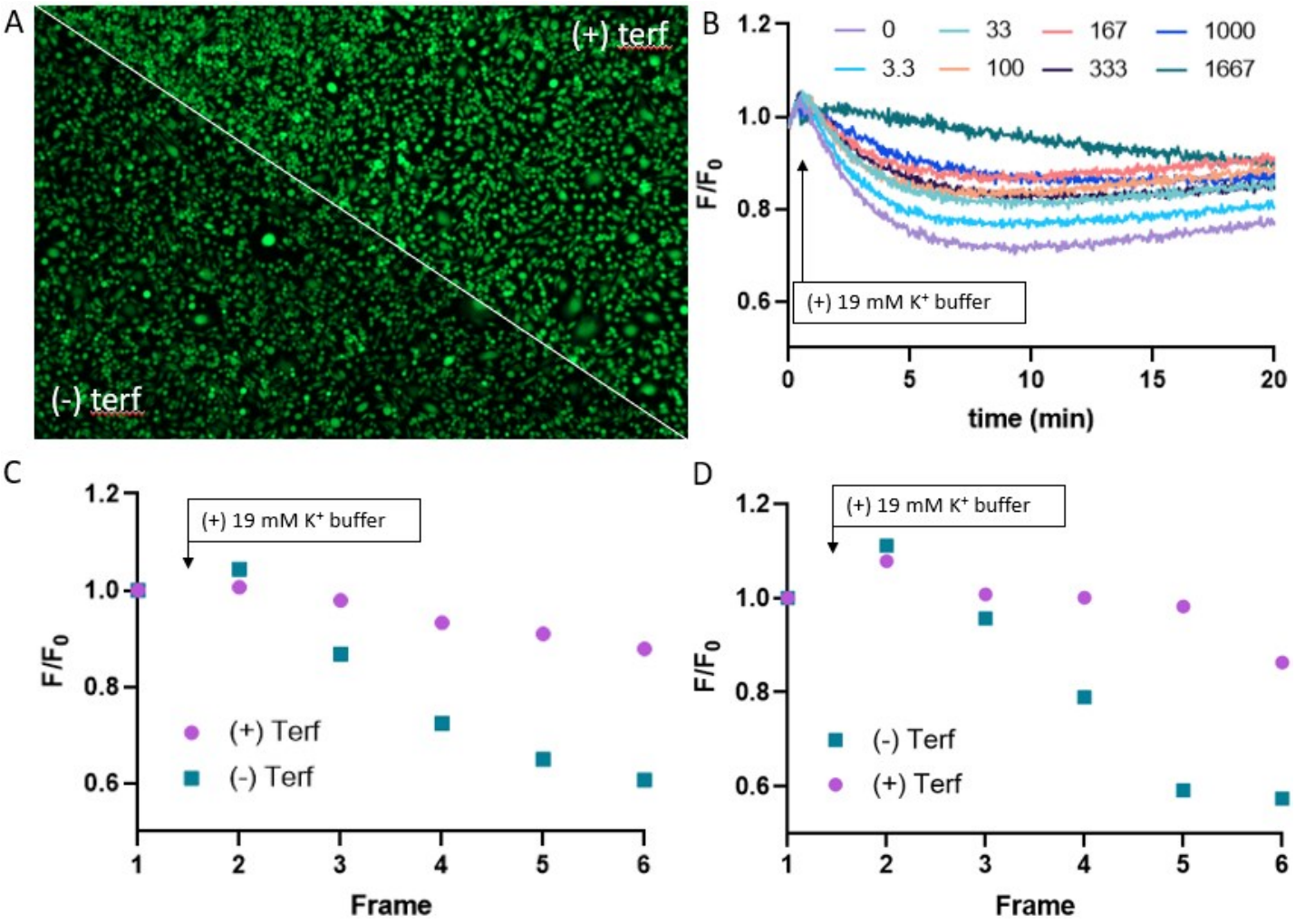


Figure 6. Evaluation of CHO hERG (K_v11.1) cells using Brilliant Thallium Snapshot Assay on a variety of instruments. A) Representative fluorescence images of cells in vehicle- (left) and the hERG inhibitor, terfenadine-treated (right) wells acquired ~9 min after the addition of a high K^+ stimulus buffer using GFP filters on a Cytation 5. The presence of terfenadine inhibits hERG activation upon membrane depolarization, effectively slowing Tl^+ efflux - which results in cells exhibiting higher fluorescence than a vehicle-treated group. B) Kinetic profiles of CHO hERG cells treated with different concentrations of terfenadine (nM). Cells were preloaded with Thallos and Tl^+(1.25 mM), then terfenadine was added 5 minutes prior to high K^+ buffer. Data was acquired using a Flexstation. C) Fluorescence images were acquired of vehicle- and terfenadine-treated (1667 nM) wells at 2-3 minute intervals, and the change in mean cell fluorescence per frame is plotted. D) Bottom read fluorescence data of vehicle- and terfenadine-treated (1667 nM) wells was acquired in concert with image frames, and the change in fluorescence over time is plotted. Data generated using both methods resembles the first 10 min of curves acquired on a Flexstation and shown in (B).

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 Mol Biol. 2018;1684:105-114.

Related Products

Product Code	Product Name
11000-100	Brilliant Thallium Assay, Express
7010T	10X Brilliant Thallium Assay Buffer
7020B	10X Brilliant Chloride-free Stimulus Buffer
7030S	10X High-Potassium, Chloride-free Stimulus Buffer
7040S	50 mM Thallium Sulfate Solution
7501A	DySolv
7060A	TRS Solution
7300P-50 (50X, 20 mL), 7300P-100 (100X, 20 mL)	Probenecid Solution