ION BIOSCIENCES

Instructions

Brilliant Thallium Gold *Snapshot*

Table 1 Package Contents

Label	Name	Volume	Containers	Storage
Reagent A	Thallos Gold AM (25 µg)	Dry	10	-20° C
Reagent B	DMSO	225 µL	1	20–25° 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 Gold 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 Gold Snapshot simultaneously loads cells with Tl^+ and Thallos Gold 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 Gold 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 Gold 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

Description

ION Biosciences Brilliant Thallium Gold 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 Gold 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 Gold 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 ~530 nm and collecting emission at ~550 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 Gold 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 100 µL of Thallium Sulfate solution (**Reagent G**) to the tube from **step 7**. While 0.5 mM thallium sulfate works well for many targets, we recommend optimizing this concentration for each assay.
9. Add 20 µL of Thallos Gold 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 Gold 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	100 µL
	Water	8.3 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 performance.*

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 1X Assay Buffer or HEPES-buffered Hank's Balanced Salt Solution (HHBS). To make 1X assay buffer, mix 9 mL of H_2O with 1 mL of 10X Thallium Snapshot Assay Buffer (**Reagent D**). 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 ~530 nm, and emission wavelength of ~546 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

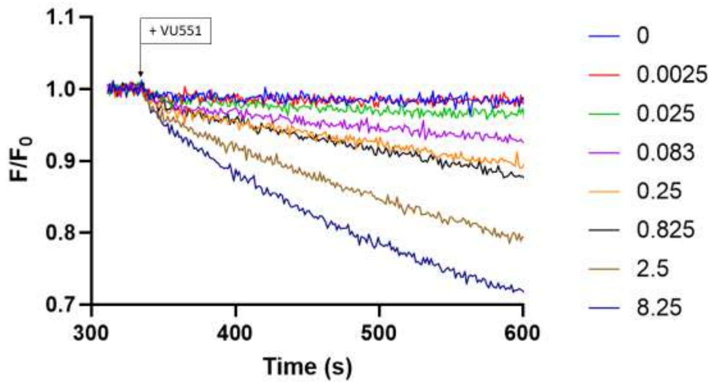
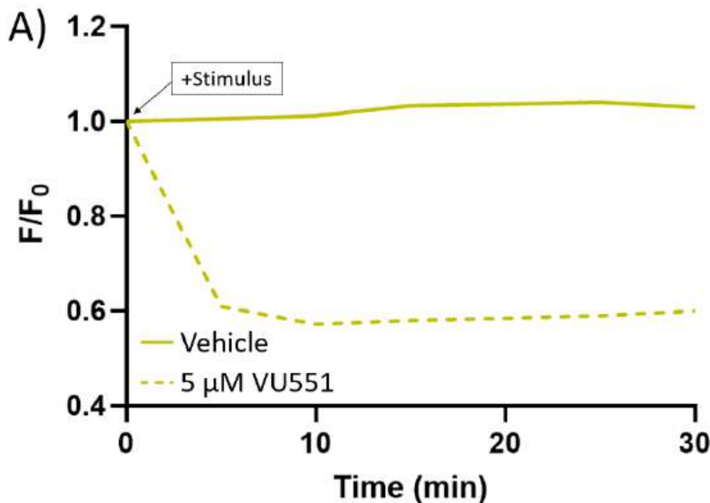


Figure 1. Kinetic fluorescence profiles of CHO GIRK1/2 (G12) cells after channel activation when using Brilliant Thallium Gold Snapshot. A) Cells are pre-loaded with Thallos Gold AM and Tl^+ prior to adding the GIRK activator, VU0466551 (VU551). Kinetic data acquired using a Flexstation. Efflux of Tl^+ upon GIRK channel activation yields a decrease in fluorescence. Legend concentrations are in μM.



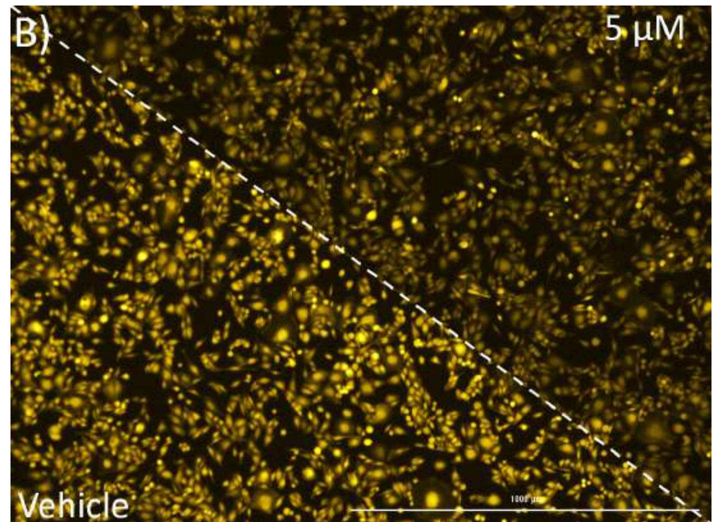


Figure 2. CHO GIRK1/2 response to a GIRK activator over time. A) Changes in signal are sustained for 30 minutes as demonstrated using a standard fluorescence plate reader (Cytation 5). Reads were acquired at 5 min intervals. B) Representative fluorescence images of vehicle- (left) and VU551-treated (right) CHO G12 cells. Images acquired 30 min after VU551 addition using propidium iodide filters and a 4X objective. A gold lookup table was applied to images.

Example Results

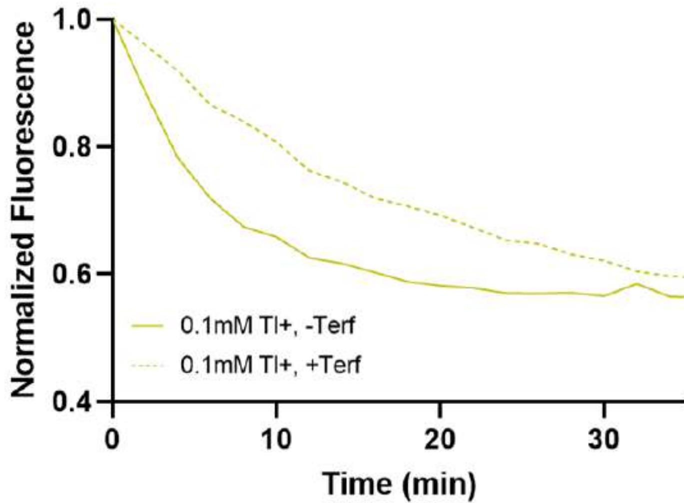


Figure 3. Evaluation of hERG (K_v11.1) inhibition using Brilliant Thallium Gold Snapshot. The presence of terfenadine inhibits hERG activation upon membrane depolarization, effectively slowing Tl^+ efflux - which results in cells exhibiting higher fluorescence than a vehicle-treated group. Cells were preloaded with Thallos Gold and Tl^+ (0.1 mM), then terfenadine (1667 nM) was added 5 minutes prior to high K^+ (19 mM) buffer. Data was acquired using a Flexstation. Signal differences were sustained

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