ION BIOSCIENCES

Instructions

Brilliant Thallium *Snapshot* Assay

snapshot compatible

Table 1	Kit Contents	*Flex* Kit Cat# 11011-10		*pIONeer* Kit Cat# 11011-2		
Label	Name	Size	Qty	Size	Qty	Storage
Reagent A	Thallos AM	25 µg Vial	10	25 µg Vial	2	-20°C
Reagent B	DMSO[1]	225 µL Vial	1	Not included in pIONeer		-20°C
Reagent C	50X DySolv	4 mL Bottle	1	800 µL Vial	1	4°C
Reagent D	10X Brilliant Thallium Snapshot Assay Buffer	10 mL Bottle	1	2 mL Bottle	1	4°C
Reagent E	50X TRS	4 mL Bottle	1	800 µL Vial	1	4°C
Reagent F	50X Probenecid	4 mL Bottle	1	800 µL Vial	1	4°C
Reagent G	50 mM Thallium Sulfate Solution	4 mL Bottle	1	800 µL Vial	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.

Description Continued

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. ION's pIONeer Kits do not contain DMSO for solubilizing the dye.

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[1] (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, next page) 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.
11. Remove the cell-culture medium from the 384-well microplate containing the cells of interest[3].
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 37° C.

Procedure Continued on Next Page

Table 2	Dye Loading Solution	Wash Method	No-Wash Method
Label	Name	Volume	Volume
Reagent A + B	Thallos AM Solution	20 µL	20 µL
Reagent C	50X DySolv	200 µL	400 µL
Reagent D	10X Brilliant Thallium Snapshot Assay Buffer	1 mL	1 mL
Reagent E	50X TRS[2]	200 µL	400 µL
Reagent F	50X Probenecid[2]	200 µL	400 µL
Reagent G	50 mM Thallium Sulfate Solution	50 µL	100 µL
	Water	8.35 mL	7.7 mL
	Total	10 mL	10 mL

14. Prepare compound solution(s) and vehicle controls at appropriate concentrations in 1X Assay Buffer or 1X HEPES-buffered Hank's Balanced Salt Solution (1X HHBSS, available from ION Biosciences, see Table 4). To make 1X assay buffer, mix 9 mL of H_2O with 1 mL of 10X Brilliant Thallium Snapshot Assay Buffer (Reagent D). We recommend a 3X concentration of compound solution(s) when using volumes suggested in this protocol. NOTE: For voltage-gated potassium channels, such as hERG (Kv11.1), 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, see Table 4.
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. See Table 3 for recommended instrument settings.
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. NOTE: Wait time will need to be optimized for each assay. We recommend conducting your initial assay in kinetic mode at a read per minute frequency to determine the optimal read time.
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 acquired.

Table 3	Recommended Instrument Settings
Setting	Recommendation
Read Mode	'Bottom' read mode only
Ex/Em wavelengths[4]	~490 nm/520 nm
Cutoff wavelength	515 nm
Filter selection	GFP or FITC
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing your application.	

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. ION's pIONeer Kits do not contain DMSO.

[2] Caution is advised when using Probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest. Probenecid may be included in the Dye Loading Solution to aid in dye retention. This may be particularly important in certain cell lines (e.g. CHO cells). TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence.

[3] 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.

[4] To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths. Results generated on a Molecular Devices Flexstation 3.

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 4	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	50X DySolv	20 mL Bottle	7501A
Reagent D	10X Brilliant Thallium Snapshot Assay Buffer	10 mL Bottle	7010T-S
Reagent E	50X TRS	20 mL Bottle	7060A
Reagent F	50X Probenecid	20 mL Bottle	7300P-50
Reagent G	50 mM Thallium Sulfate	5 mL Bottle	7040S
Step 14	1X HEPES-Buffered Hanks Balanced Salt Solution (HHBSS)	100 mL Bottle	7001
Step 14	10X High-Potassium (K^+), Chloride-Free Stimulus Buffer	10 mL Bottle	7030S