Brilliant Thallium *Flex*

Table 1	Package Contents			
Label	**Name**	**Volume**	**Containers**	**Storage**
Reagent A	Brilliant Thallium Reagent	Dry	10	-20° C
Reagent B	DMSO	225 µL	1	4° C
Reagent C	DySolv	4 mL	1	4° C
Reagent D	10X Assay Buffer	20 mL	1	4° C
Reagent E	TRS	4 mL	1	4° C
Reagent F	Probenecid Solution	4 mL	1	4° C
Reagent G	10X Chloride-Free Stimulus Buffer	10 mL	1	4° C
Reagent H	10X High-Potassium Stimulus Buffer	10 mL	1	4° C
Reagent J	50 mM Thallium Sulfate Solution	20 mL	1	20–25° C

Description

ION Biosciences Brilliant Thallium 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. The ION Biosciences Brilliant Thallium 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 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. 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 Brilliant Thallium *Flex* provides all the reagents necessary for use as a washed or 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 cells 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.

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 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 ION Brilliant Thallium Assay package. Notably compounds to be tested are not included, neither are buffers and solvents for the dissolution of those compounds. The Brilliant Thallium Assay package also does not contain reagents necessary for cell culture.

In addition to reagents, a fluorescence plate reader that is capable of providing excitation at ~ 490 nm and collecting emission at ~ 520 nM is required. Ideally this plate reader will be able to collect kinetic data at an interval of once per second (1 Hz). Examples of plate readers of this type are the WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR and Molecular Devices FlexStation.

Wash Method — Adherent Cells

The instructions given below are 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). The ION Brilliant Thallium indicator and ION Brilliant Thallium indicator-containing solutions should be protected from direct light.

1. Add 20 µL DMSO (**Reagent B**) to the tube containing ION Brilliant Thallium indicator (**Reagent A**)
2. Vortex until Reagent A is fully dissolved.

Table 2 Dye Loading Solution

Label	Name	Method A	Method B
Reagent A	Brilliant Thallium Indicator Solution	20 µL	20 µL
Reagent C	DySolv	200 µL	200 µL
Reagent D	10X Assay Buffer	1 mL	1 mL
Reagent F	Probenecid Solution*	-	200 µL
	Water	8.8 mL	8.6 mL
	Total	10 mL	10 mL

**Probenecid may be included in the 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 for the target of interest.*

3. Add appropriate volume of water (**Table 2**) to a 15 mL centrifuge tube.
4. Add 1 mL of 10X Assay Buffer (**Reagent D**) to tube from **step 3**.
5. Add 200 µL of DySolv (**Reagent C**) to the tube from **step 4**.
6. If desired add 200 µL of Probenecid Solution (**Reagent F)** to the tube from **step 5**.
7. Add 20 µL of Brilliant Thallium Indicator Solution from **step 2** to the tube from **step 6**.
8. Briefly vortex the tube from **step 7** to mix.
9. Remove the cell-culture medium from the 384-well microplate containing the cells of interest.
10. Add 20 µL per well of the Dye Loading Solution from **step 8** to the microplate from **step 9**.
11. Incubate the microplate containing the cells and Dye Loading Solution for 1 hour at room temperature.

Table 3 Wash Solution

Label	Name	Method A	Method B	Method C	Method D
Reagent D	10X Assay Buffer	1 mL	1 mL	1 mL	1 mL
Reagent E	TRS*	-	200 µL	-	200 µL
Reagent F	Probenecid Solution	-	-	200 µL	200 µL
Reagent H	Water	9 mL	8.8 mL	8.8 mL	8.6 mL
	Total	10 mL	10 mL	10 mL	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.*

12. Prepare Wash Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Assay Buffer (**Reagent D**) and other components if desired as shown in **Table 3**.
13. Briefly vortex the tube from **step 12** to mix.
14. Remove Dye Loading Solution from microplate in **step 11**.
15. Add 20 µL per well of the Wash Solution prepared in **step 13** to the microplate from **step 14**.
16. Prepare Thallium Stimulus Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Stimulus Buffer (**Reagents G/H**) and Thallium Sulfate Solution (**Reagent J**) as shown in **Table 4***.
17. Briefly vortex the tube from **step 16** to mix.
18. Add 20 µL per well of the Thallium Stimulus Solution from **step 17** to an empty 384-well microplate.

Table 4	Thallium Stimulus Solution*		
Label	**Name**	**Example A**	**Example B**
Reagent G	10X Chloride-Free Stimulus Buffer	1 mL	0.5 mL
Reagent H	10X High-Potassium Stimulus Buffer	-	0.5 mL
Reagent J	50 mM Thallium Sulfate Solution	0.5 mL	0.5 mL
	Water	8.5 mL	8.5 mL
	Total	10 mL	10 mL

**__Table 4__ provides two examples of Thallium Stimulus solutions useful for many types of non-voltage-gated and voltage-gated monovalent cation channels and transporters. Elevation of extracellular potassium (__Example B__) may provide superior results for some voltage-gated channels. The concentration of thallium in the stimulus solution may be varied to achieve the desired result. The final thallium concentration in the cell-containing microplate post-thallium stimulus buffer addition should not exceed 4.8 mM due to the ~ 5 mM solubility limit of thallium in chloride-containing solutions.*

19. Transfer the washed, dye-loaded, cell-containing microplate from **step 15** and the Thallium Stimulus Solution microplate from **step 17** to a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR or Molecular Devices FlexStation).
20. Acquire data using an excitation wavelength of ~ 490 nm, an emission wavelength of ~ 520 nm and an acquisition frequency of 1 Hz. Begin data acquisition and after 10 seconds add 5 µL of the Thallium Stimulus Solution to the cell-containing plate and continue data acquisition for an additional 90 seconds**.

***The timing of and volume of Thallium Stimulus Solution addition may vary. In some cases, experiments may include the addition of other solutions to the cell-containing microplate prior to the addition of the Thallium Stimulus Solution. In these cases, the volume of the Thallium Stimulus Solution addition should be altered to account for the additional volume of solution in the cell-containing microplate.*

No-wash Method — Adherent Cells

1. Add 20 µL DMSO (**Reagent B**) to the tube containing ION Brilliant Thallium indicator (**Reagent A**)
2. Vortex until Reagent A is fully dissolved.

Table 5 Dye Loading Solution

Label	Name	Method A	Method B
Reagent A	Brilliant Thallium Indicator Solution	20 µL	20 µL
Reagent C	DySolv	400 µL	400 µL
Reagent D	10X Assay Buffer	1 mL	1 mL
Reagent E	TRS*	400 µL	400 µL
Reagent F	Probenecid Solution**	-	400 µL
	Water	8.2 mL	7.8 mL
	Total	10 mL	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 the 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 for the target of interest.*

3. Add appropriate volume of water (**Table 5**) to a 15 mL centrifuge tube.
4. Add 1 mL of 10X Assay Buffer (**Reagent D**) to tube from **step 3**.
5. Add 400 µL of DySolv (**Reagent C**) to the tube from **step 4**.
6. Add 400 µL of TRS (**Reagent E**) to the tube from **step 5**.
7. If desired add 400 µL of Probenecid Solution (**Reagent F**) to the tube from **step 6**.
8. Add 20 µL of Brilliant Thallium Indicator Solution from **step 2** to the tube from **step 7**.
9. Briefly vortex the tube from **step 8** to mix.
10. Add 20 µL per well of the Dye Loading Solution from **step 9** to the cell-containing microplate. Do not remove the cell culture medium.
11. Incubate the microplate containing the cells and Dye Loading Solution for 1 hour at 37° C in a cell culture incubator.

Table 6	Thallium Stimulus Solution*		
Label	**Name**	**Example A**	**Example B**
Reagent G	10X Chloride Free Stimulus Buffer	1 mL	0.5 mL
Reagent H	10X High Potassium Stimulus Buffer	-	0.5 mL
Reagent J	50 mM Thallium Sulfate Solution	0.5 mL	0.5 mL
	Water	8.5 mL	8.5 mL
	Total	10 mL	10 mL

Table 6 provides two examples of Thallium Stimulus solutions useful for many types of non-voltage-gated and voltage-gated monovalent cation channels and transporters. Elevation of extracellular potassium (Example B**) may provide superior results for some voltage-gated channels. The concentration of thallium in the stimulus solution may be varied to achieve the desired result. The final thallium concentration in the cell-containing microplate post-thallium stimulus buffer addition should not exceed 4.8 mM due to the ~ 5 mM solubility limit of thallium in chloride-containing solutions.*

12. Prepare Thallium Stimulus Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Stimulus Buffer (**Reagents G/H**) and Thallium Sulfate Solution (**Reagent J**) as shown in **Table 6***.
13. Briefly vortex the tube from **step 12** to mix.
14. Add 20 µL per well of the Thallium Stimulus Solution from **step 13** to an empty 384-well microplate.
15. Transfer the dye-loaded, cell-containing microplate from **step 11** and the Thallium Stimulus Solution microplate from **step 14** to a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR or Molecular Devices FlexStation).
16. Acquire data using an excitation wavelength of ~ 490 nm, an emission wavelength of ~ 520 nm and an acquisition frequency of 1 Hz. Begin data acquisition and after 10 seconds add 10 µL of the Thallium Stimulus Solution to the cell-containing plate and continue data acquisition for an additional 90 seconds**.

***The timing of and volume of Thallium Stimulus Solution addition may vary. In some cases, experiments may include the addition of other solutions to the cell-containing microplate prior to the addition of the Thallium Stimulus Solution. In these cases, the volume of the Thallium Stimulus Solution addition should be altered to account for the additional volume of solution in the cell-containing microplate.*

Example Results

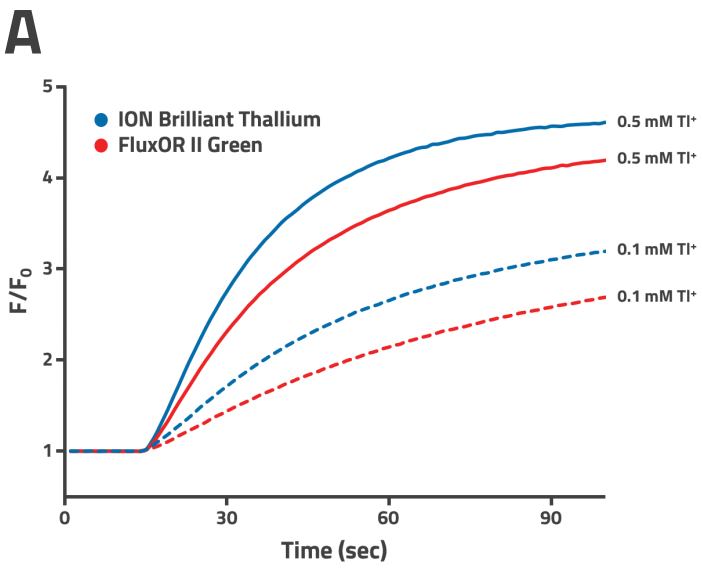

Figure 1. Tl^+ Sensitivity in Wash and No-Wash Formats . HEK-293 cells expressing an inward rectifying K^+ channel were tested with either ION Brilliant Thallium (**blue**) or FluxOR II Green (**red**) in no-wash mode (**A**) or washed mode (**B**) using the manufacturer's instructions. Dye-loaded cells were exposed to either 0.1 mM or 0.5 mM Tl^+.

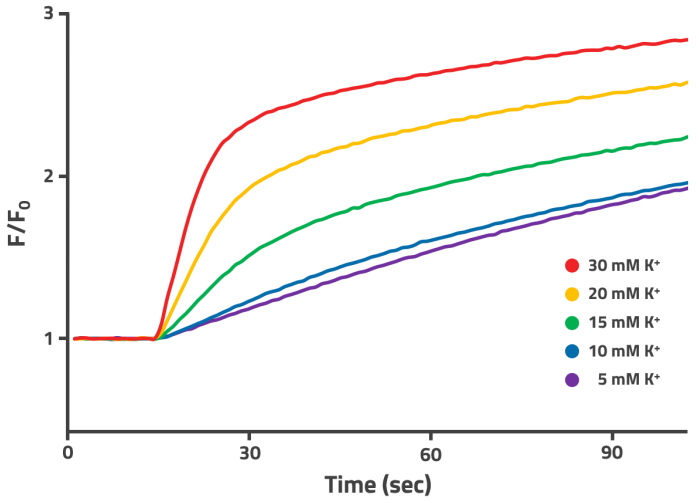

Figure 2. Effect of Varying Extracellular K^+ Concentration on Voltage-activated Channel. HEK-293 cells expressing a voltage-activated K^+ channel were tested using ION Brilliant Thallium in washed mode. Cells were then exposed to 1 mM Tl^+ and varying concentrations of K^+ ranging from 5– 30 mM.

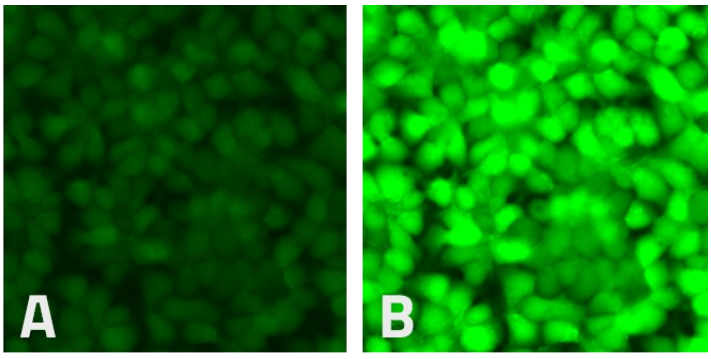

Figure 3. Tl^+-evoked Changes in Cellular Fluorescence. HEK-293 cells expressing a voltage-activated K^+ channel, loaded with ION Brilliant Thallium Assay reagent before (**A**) and after (**B**) exposure to Tl^+.