ION BIOSCIENCES

Instructions

Brilliant Thallium Gold *Flex*

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	4—25° 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 ($[K^+]$ = 0 M, $[Na^+]$=	10 mL	1	4° C
Reagent H	10X High-Potassium Stimulus Buffer ($[K^+]$ = 1.38 M,	10 mL	1	4° C
Reagent J	50 mM Thallium Sulfate Solution	20 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 Assay kit 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 kit includes our proprietary Thallos Gold indicator (Ex/Em: 530/546 nm), which is responsive to intracellular Tl^+ dynamics. Thallos Gold's spectral properties allow for multiplexing with GFP-expressing cells or other green-fluorescent indicators, and will help mitigate false positives caused by auto-fluorescent compounds within a library. The ION Biosciences Brilliant Thallium Gold 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 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. ION Brilliant Thallium Gold *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 Gold 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 Gold Assay package. Notably compounds to be tested are not included, neither are buffers and solvents for the dissolution of those compounds. The Brilliant Thallium Gold 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 ~ 530 nm and collecting emission at ~ 550 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 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 Thallos Gold AM and Thallos Gold AM-containing solutions should be protected from direct light.

1. Add 20 µL DMSO (**Reagent B**) to the tube containing Thallos Gold AM (**Reagent A**)
2. Vortex until Reagent A is fully dissolved.

Table 2 Dye Loading Solution

Label	Name	Method A	Method B
Reagent A	Thallos Gold AM 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 Thallos Gold AM 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**.

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 desired components 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 ($[K^+]$ = 0 M, $[Na^+]$= 1.38 M)	1 mL	0.5 mL
Reagent H	10X High-Potassium Stimulus Buffer ($[K^+]$ = 1.38 M, $[Na^+]$ = 0 M)	-	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 ~530 nm, an emission wavelength of ~550 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 Thallos Gold AM (**Reagent A**)
2. Vortex until Reagent A is fully dissolved.

Table 5 Dye Loading Solution

Label	Name	Method A	Method B
Reagent A	Thallos Gold AM 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*

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 Thallos Gold AM 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 ($[K^+]$ = 0 M, $[Na^+]$= 1.38 M)	1 mL	0.5 mL
Reagent H	10X High-Potassium Stimulus Buffer ($[K^+]$ = 1.38 M, $[Na^+]$ = 0 M)	-	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 ~ 530 nm, an emission wavelength of ~ 550 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

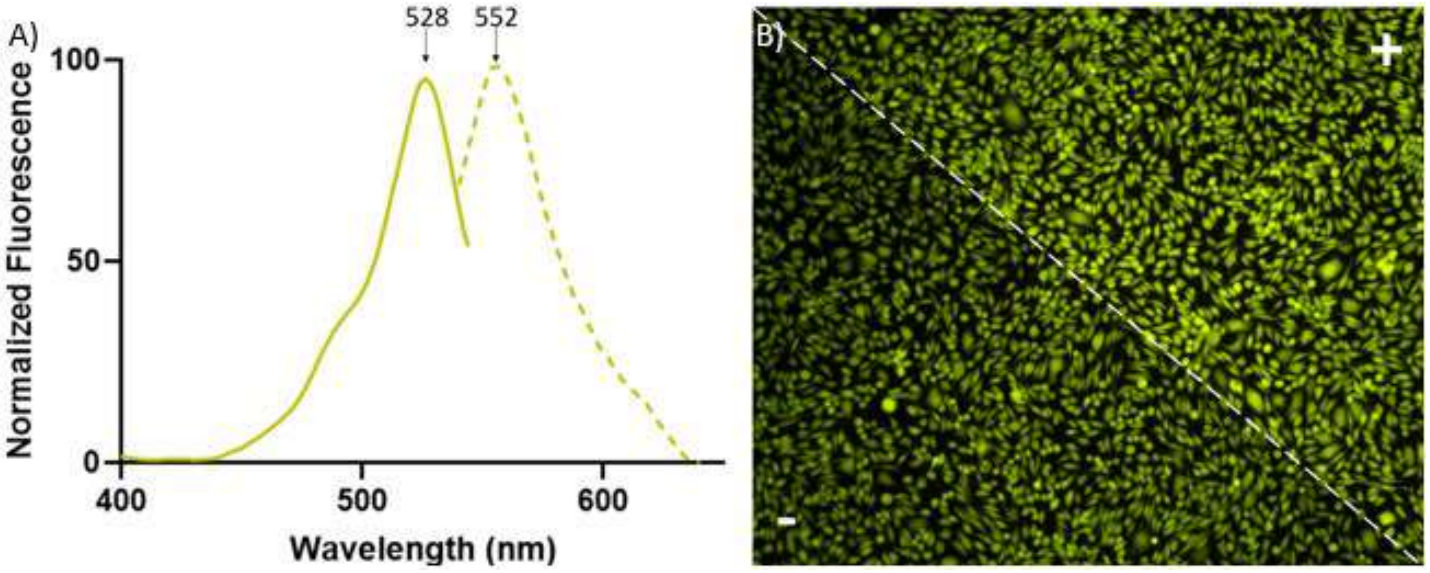

Figure 1. Spectral properties of Thallos Gold. CHO K1 cells were loaded with Thallos Gold for 30 minutes before data acquisition. A) Spectral data was acquired with a BioTek® Cytation 5 plate reader. Maximum excitation is 528 nm and maximum emission is 552 nm. B) Cells were imaged using a Zeiss Axiovert 25 Inverted Phase Contrast Microscope, before (-) and after (+) the addition of 0.83mM Tl_2SO_4 (Ex: 517/20 nm, Em: 575/59 nm).

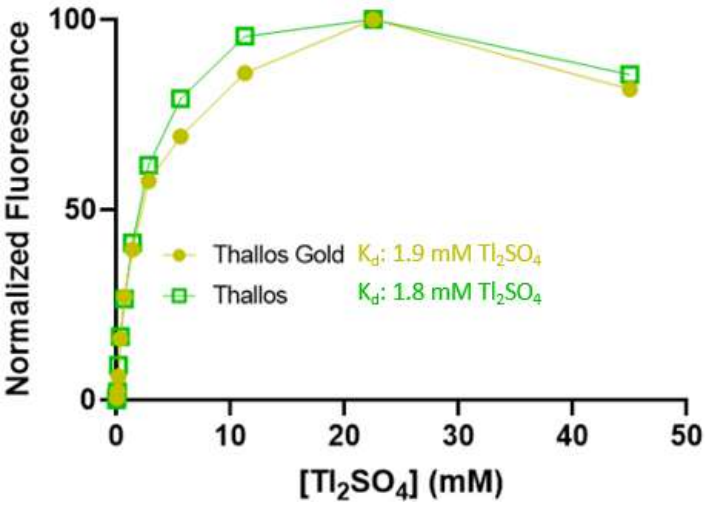

Figure 2. Saturation binding curves. Thallos and Thallos Gold titrations in gluconate buffers. TMACl was included to maintain a constant ionic strength (300 mOsm). Similar K_d values are obtained.

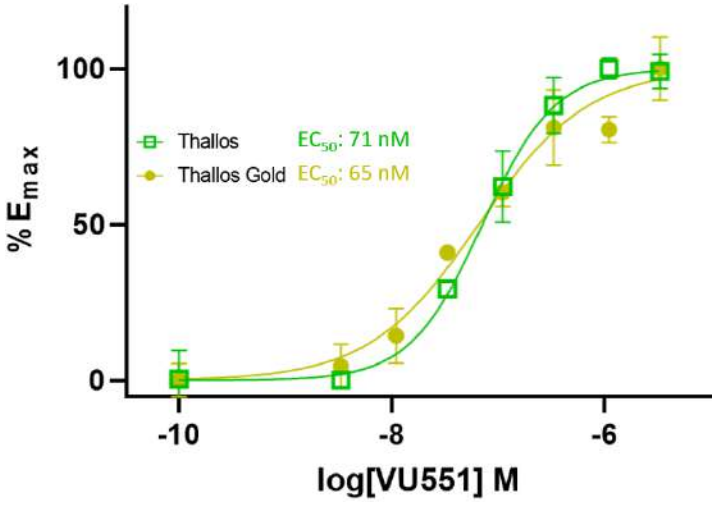

Figure 3. Thallos and Thallos Gold response. Concentration response curve of VU551 in CHO cells overexpressing GIRK channels. VU551 is a potent activator of GIRK1/2, resulting in an increased flux of Tl^+. Green fluorescence (Ex: 485 nm, Em: 525 nm, Cutoff: 515 nm) and gold fluorescence (Ex: 520 nm, Em: 555 nm, Cutoff: 550 nm) were recorded at ~1 Hz using a Molecular Devices FlexStation®. Error bars represent standard deviation (n=3).

Example Results

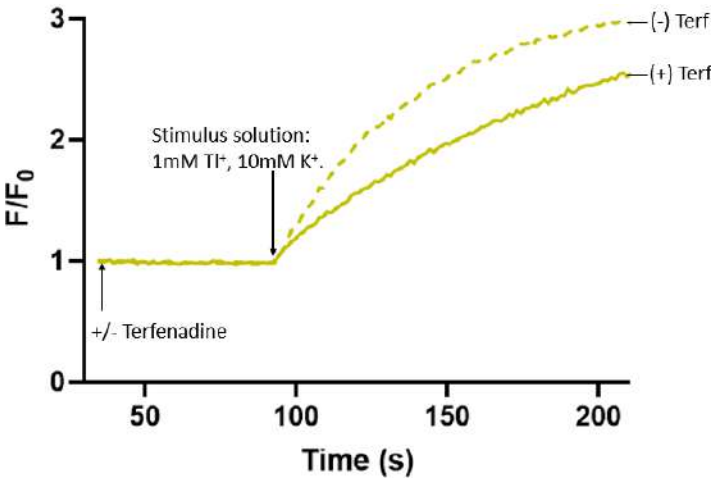

Figure 4. Effect of inhibitor on voltage-activated channel. HEK-293 cells expressing the hERG channel (K_v11.1), a voltage-activated K^+ channel, were loaded with Thallos Gold for 1 hour. Fluorescence profiles were acquired on a Molecular Devices FlexStation® (Ex: 520 nm, Em: 555 nm, Cutoff: 550 nm) for three minutes. One minute after the addition of terfenadine (490 nM), an inhibitor of the hERG channel, and 2 minutes after activating the hERG channel with a high K^+ (10 mM), Tl^+ (1 mM) stimulus solution. Concentrations reported are final concentrations.

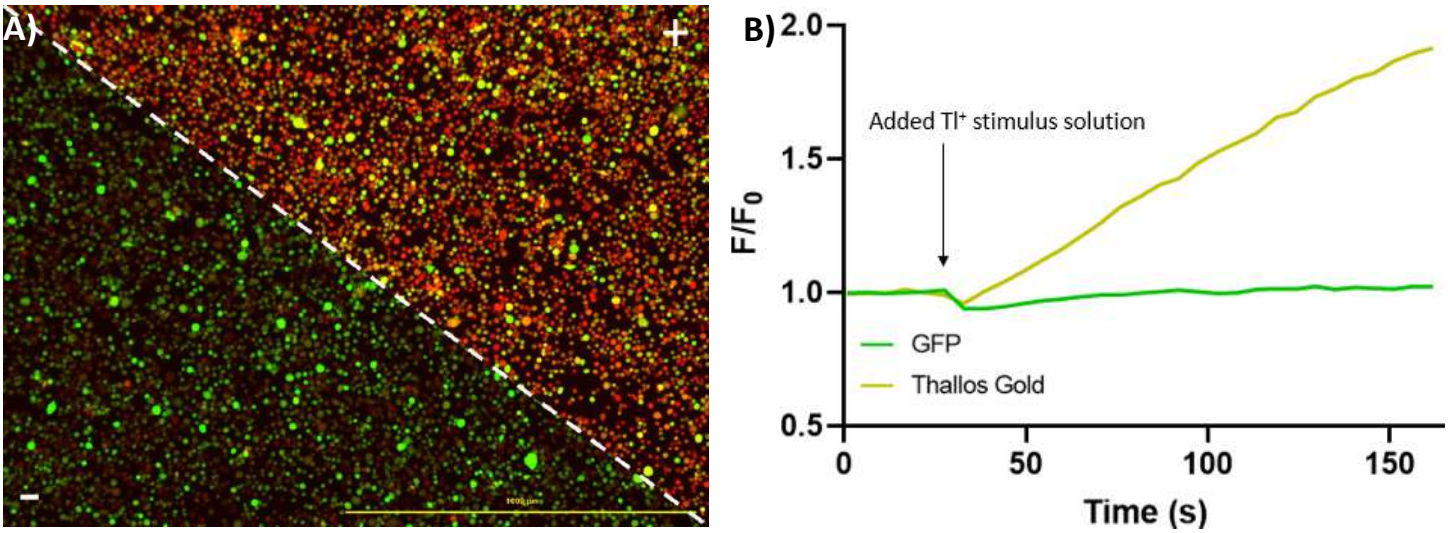

Figure 5. Thallium flux in GFP-expressing cells loaded with Thallos Gold. A) Representative fluorescence images of GFP-expressing CHO cells loaded with Thallos Gold for ~30 minutes. Images were acquired before (-) and after (+) addition of the thallium stimulus solution with a BioTek® Cytation equipped with a GFP filter cube (Ex: 469/35 nm, Em: 529/39 nm), Propidium Iodide filter cube (Ex: 531/40 nm, Em: 647/57 nm)*, and 4X objective. Image analysis shows a 2.2 fold change in fluorescence for Thallos Gold. B) Kinetic data showing fold change over the span of two minutes after the addition of Tl^+ stimulus solution (0.83mM Tl_2SO_4) to CHO cells expressing GFP and loaded with Thallos Gold. Green fluorescence (Ex: 485 nm, Em: 525 nm, Cutoff: 515 nm) and gold fluorescence (Ex: 520 nm, Em: 555 nm, Cutoff: 550 nm) were recorded at ~1 Hz using a Molecular Devices FlexStation® plate reader. *Thallos Gold appears red in images because a Propidium Iodide filter cube was used.

Example Results

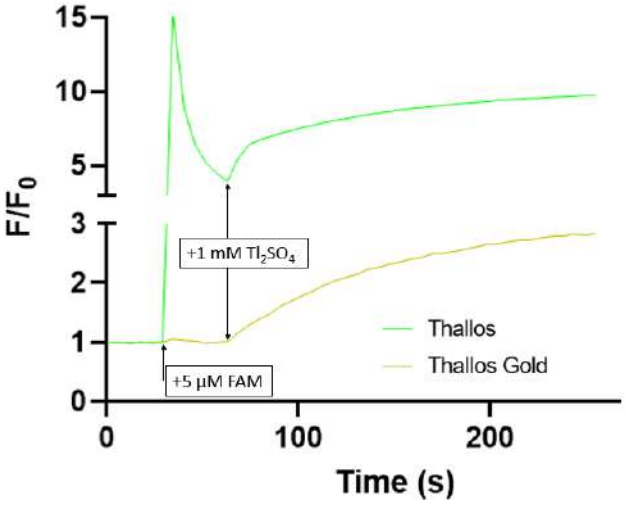

Figure 6. Thallos Gold minimizes compound interference from auto-fluorescent compounds. CHO K1 cells were loaded with Thallos and Thallos Gold in separate wells for 30 minutes. Acquisition settings for Thallos (Ex: 485 nm, Em: 525 nm, Cutoff: 515 nm) and Thallos Gold (Ex: 540 nm, Em: 580 nm, Cutoff: 570 nm) were recorded at ~1 Hz using a Molecular Devices FlexStation®. Baseline fluorescence was collected for 30 seconds before the addition of 5 µM FAM, a highly fluorescent compound. FAM fluorescence does not disrupt Thallos Gold signal. Thallium stimulus solution was added 30 seconds after the addition of FAM.

References

1. Dutter, et al. Rhodol-based Thallium Sensors for Cellular Imaging of Potassium Channel Activity. Org Biomol Chem. 2018 Aug 8; 16(31): 5575-5579.
2. McClenahan, et al. VU6036720: The First Potent and Selective In Vitro Inhibitor of Heteromeric Kir4.1/5.1 Inward Rectifier Potassium Channels. Mol Pharmacol. 2022 May;101(5):357-370.
3. 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