THALLOS GOLD AM

Materials Needed

Name	Volume	Containers	Storage
Thallos Gold AM (50 µg vial)	Dry	1	-20° C
DMSO	25 µL	1	20—25° C
100X Pluronic F-127 solution	100 µL	1	4° C
100X Probenecid solution (optional)	100 µL	1	4° C
100X TRS solution (optional)	100 µL	1	4° C
10X Chloride-Free Stimulus Buffer	10 mL	1	4° C
10X High-Potassium Stimulus Buffer	10 mL	1	4° C
50 mM Thallium Sulfate Solution	20 mL	1	20—25° C

Description

Thallos Gold is a gold fluorescent (Ex/Em = 528/552 nm) intracellular thallium (Tl^+) indicator. Thallos Gold delivers outstanding results for fluorescence-based potassium (K^+) channel HTS and for a wide variety of monovalent cation (sodium - Na^+) channels, transporters, and GPCRs. Red-shifted excitation and emission enable multiplexing with GFP-expressing cells or other green fluorescent indicators and minimizes interference from auto-fluorescent compounds.

Laboratory Procedures

The following protocol provides general guidelines for using this dye to measure intracellular thallium flux. All loading conditions (dye concentration, temperature, and time) should be optimized for your specific assay and instrumentation.

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 10 mL of assay buffer to a conical tube (15 – 50 mL). HEPES-buffered Hank's Balanced Salt Solution (pH = 7.2 – 7.4) is the most used assay buffer, although other buffers can also be used. We recommend using our 1X Brilliant Thallium assay buffer (Catalog #: 7050s) with this product.
3. Add 100 µL of 100X Pluronic F-127 solution (Catalog #: 7601A) to conical tube. Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.
4. (Optional) Add 100 µL of 100X Probenecid solution (Catalog #: 7300A) to conical tube. Probenecid is an anion transport inhibitor that improves intracellular dye retention. Although it is not required for all cell types and dyes, it is recommended in most cases to optimize assay performance.[1]

[1]Caution is advised when using probenecid as it may have undesirable effects on assay performance for the your target.

Laboratory Procedures (continued)

5. (Optional) Add 100 µL of TRS solution (Catalog #: 7060A). TRS is a membrane impermeant dye useful for masking extracellular fluorescence.[1]
6. Vortex conical tube briefly to mix.
7. Dissolve Thallos Gold AM in 25 µL of DMSO. After adding DMSO, vortex tube briefly to dissolve the dye, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of dye tube to assay buffer solution to make a dye loading solution.[2]
8. Vortex dye loading solution briefly to mix.
9. Remove the cell culture medium from your cells and add dye loading solution. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.[3]
10. Incubate in a cell culture incubator at 37ºC for 30-60 minutes.
11. Prepare Thallium Stimulus Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Stimulus Buffer (Catalog #: 7020B) and Thallium Sulfate Solution (Catalog #: 7040S).
12. 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**.[4]

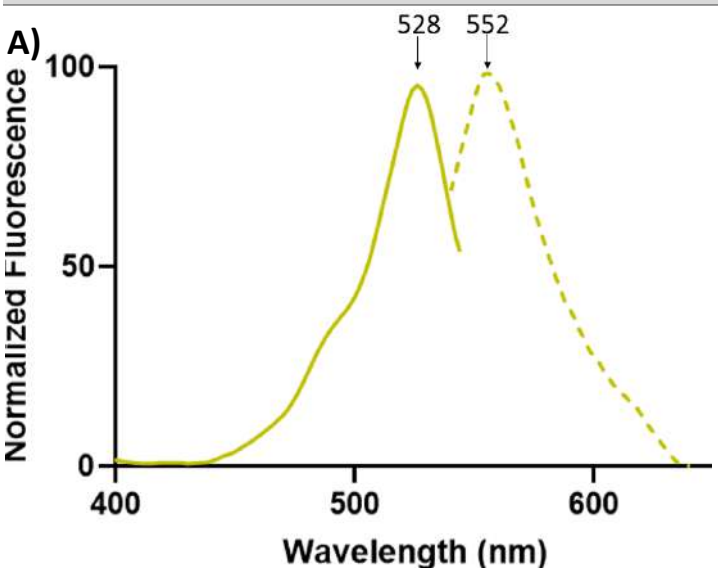
[1]Caution is advised when using TRS as it may have undesirable effects on assay performance for the your target.

[2]The dye loading solution should be used within 2 hours of dye addition for best results.

[3]In some cases, a no wash format works best. If a no wash format is indicated for your application, we recommend doubling the concentration of all reagents in your dye loading buffer.

[4]To minimize extracellular background, dye loading solution can be replaced with assay buffer containing 1X probenecid solution (optional) and/or 1X TRS solution (optional).

Example Results



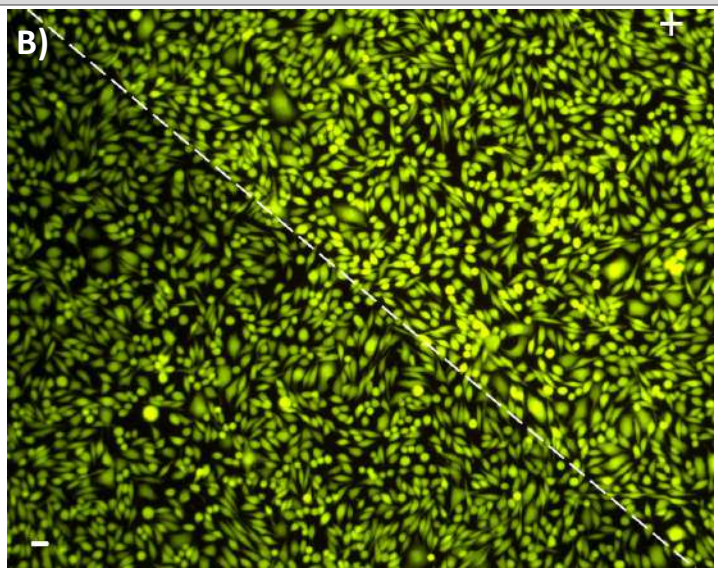


Figure 1. Spectral properties of Thallos Gold. CHO K1 cells were loaded with Thallos Gold for 30 minutes before data acquisition. A) Spectral data was acquired with a BioTek® Cytation 5 plate reader. Maximum excitation is 528 nm and maximum emission is 552 nm. B) Cells were imaged using a Zeiss Axiovert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

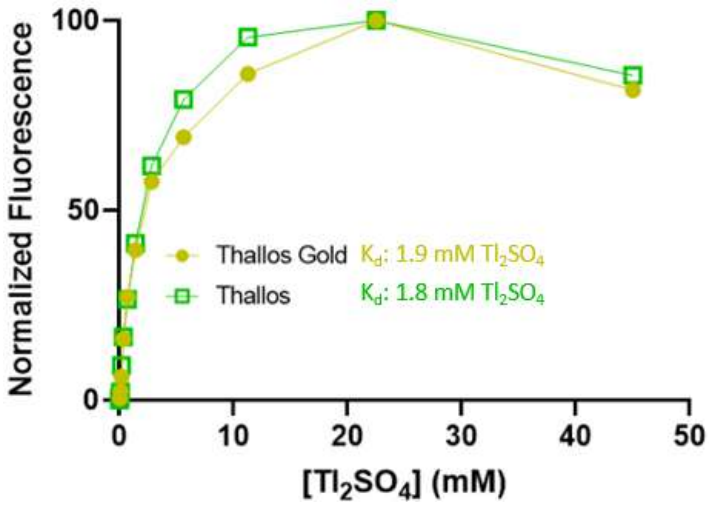


Figure 2. Saturation binding curves. Thallos and Thallos Gold titrations in gluconate buffers. TMACl was included to maintain a constant ionic strength (300 mOsm). Similar K_d values are obtained.

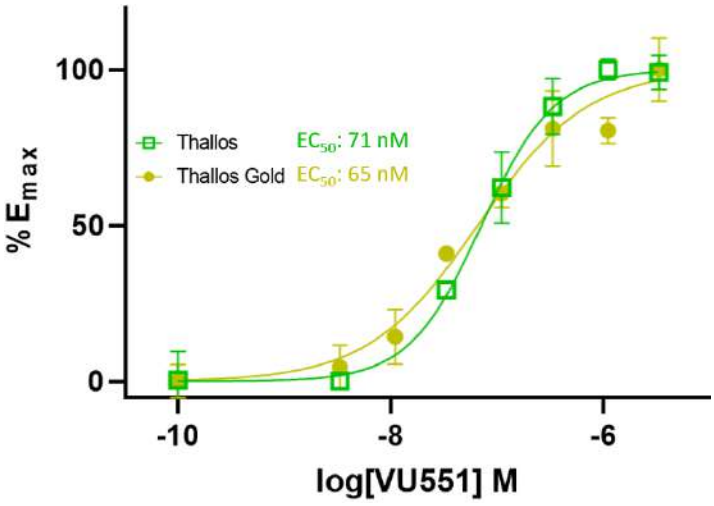


Figure 3. Thallos and Thallos Gold response. Concentration response curve of VU551 in CHO cells overexpressing GIRK channels. VU551 is a potent activator of GIRK1/2, resulting in an increased flux of Tl^+. Green fluorescence (Ex: 485 nm, Em: 525 nm, Cutoff: 515 nm) and gold fluorescence (Ex: 520 nm,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

Example Results

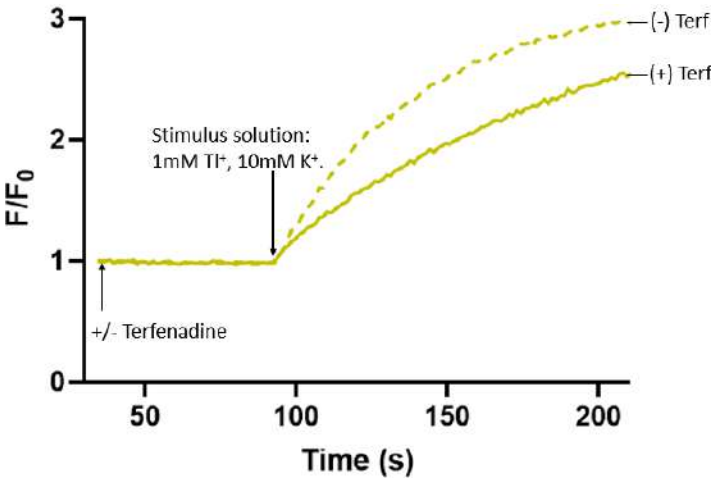


Figure 4. Effect of inhibitor on voltage-activated channel. HEK-293 cells expressing the hERG channel (K_v11.1), a voltage-activated K^+ channel, were loaded with Thallos Gold for 1 hour. Fluorescence profiles were acquired on a Molecular Devices FlexStation® (Ex: 520 nm, Em: 555 nm, Cutoff: 550 nm) 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.

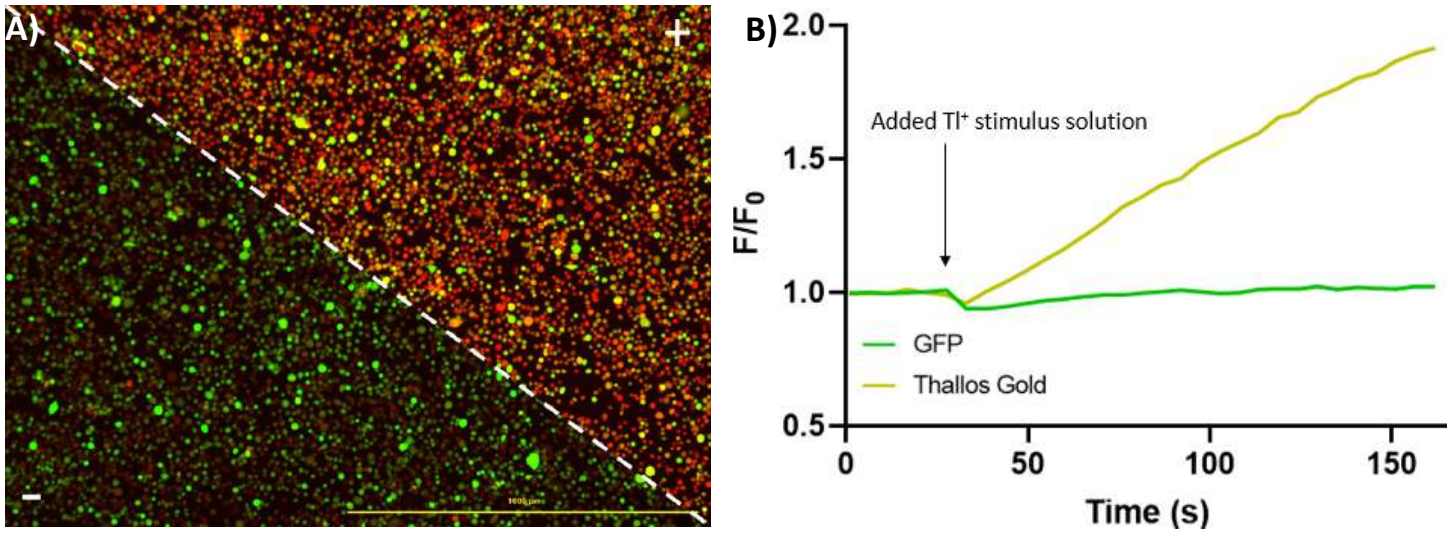


Figure 5. Thallium flux in GFP-expressing cells loaded with Thallos Gold. A) Representative fluorescence images of GFP-expressing CHO cells loaded with Thallos Gold for ~30 minutes. Images were acquired before (-) and after (+) addition of the thallium stimulus solution with a BioTek® Cytation equipped 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

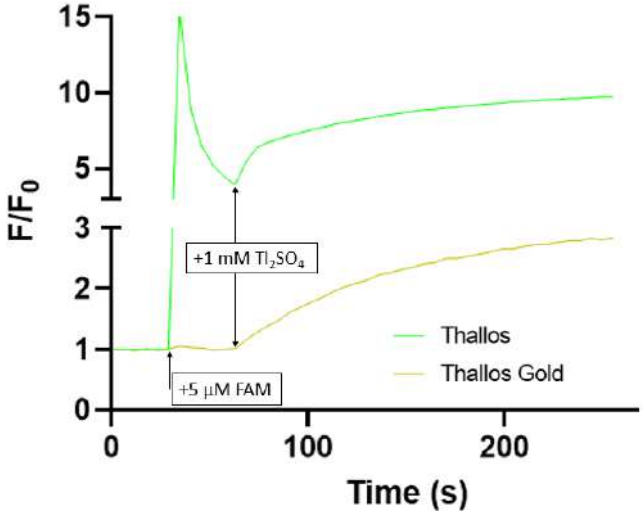


Figure 6. Thallos Gold minimizes compound interference from auto-fluorescent compounds. CHO K1 cells were loaded with Thallos and Thallos Gold in separate wells for 30 minutes. Acquisition settings for Thallos (Ex: 485 nm, Em: 525 nm, Cutoff: 515 nm) and Thallos Gold (Ex: 540 nm, Em: 580 nm, Cutoff: 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