

ION Thallos-HTL

Materials Needed

Name	Volume	Containers	Storage
ION Thallos-HTL (25 µg vial)	Dry	10	-20° C
DMSO	25 µL	1	25° C
Assay Buffer (HHBSS, etc)	10 mL	1	4° 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

Description

Thallos is a green fluorescent, intracellular thallium (Tl^+) indicator, and has been the gold standard for fluorescence-based potassium (K^+) channel HTS for nearly 2 decades. **ION Thallos-HTL** offers the opportunity to localize a thallium indicator to individual cells within a mixed cell population or intracellular compartments using HaloTag® expression technology, without compromising its Tl^+ sensitivity. Once **ION Thallos-HTL** is loaded into cells expressing HaloTag®, the indicator covalently binds to the protein. Addition of a thallium stimulus solution will show ion flux within the cell type or intracellular compartment of interest. ***This dye requires the use of cells transiently or stably expressing HaloTag® to work.** For more information about HaloTag® technology, visit Promega.com.

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, cell type, and application. Whether a wash step to remove the dye loading solution is needed, will depend on cell type. ***This dye requires the use of cells transiently or stably expressing HaloTag® to work.**

Dye Load With Wash Protocol (Recommended for CHO and HeLa cells)

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.

Laboratory Procedures (continued)

4. Add 100 μ L 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
5. (Optional) Add 100 μ L 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 **ION Thallos HTL** in 25 μ L of DMSO. After adding DMSO, vortex tube briefly to dissolve the indicator, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of indicator solution to assay buffer solution prepared in steps 2-5 to make a dye loading solution.²
8. Vortex dye loading solution briefly to mix.
9. Remove the cell culture medium and add dye loading solution. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 μ L; 384-well plate, 20 μ L.
10. Incubate in a cell culture incubator at 37°C for 60 minutes.
11. Make the wash solution with 10 mL of assay buffer and (optional) 100 μ L of 100X TRS solution. **Do NOT add Probenecid to the wash solution.
12. After the 60 minute incubation period, wash wells, incubate at 37°C for 30 minutes. **If TRS is not included in the initial wash solution in step 11, make sure to wash wells again just before proceeding to the next step.
13. Read fluorescence using a plate reader (Ex/Em: 490 nm/515 nm) or image using a fluorescence microscope (using filters for fluorescein or GFP).

Dye Load, No Wash Protocol (Recommended for HEK293 cells)

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 μ L 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 μ L of TRS solution (Catalog #: 7060A). TRS is a membrane impermeant dye useful for masking extracellular fluorescence.¹
5. Vortex conical tube briefly to mix.
6. Dissolve **ION Thallos-HTL** in 25 μ L of DMSO. After adding DMSO, vortex tube briefly to dissolve the indicator, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of indicator solution to assay buffer solution prepared in steps 2-4 to make a dye loading solution.²
7. Vortex dye loading solution briefly to mix.

Laboratory Procedures (continued)

8. Remove cell culture medium and add dye loading solution. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 μ L; 384-well plate, 20 μ L.
9. Incubate in a cell culture incubator at 37°C for 1–2 hours.
10. Read fluorescence using a plate reader (Ex/Em: 490 nm/515 nm) or image using a fluorescence microscope (using filters for fluorescein or GFP).³

¹Caution is advised when using probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest.

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

³If extracellular background signal is high, dye loading solution can be replaced with assay buffer containing 1X TRS solution (optional).

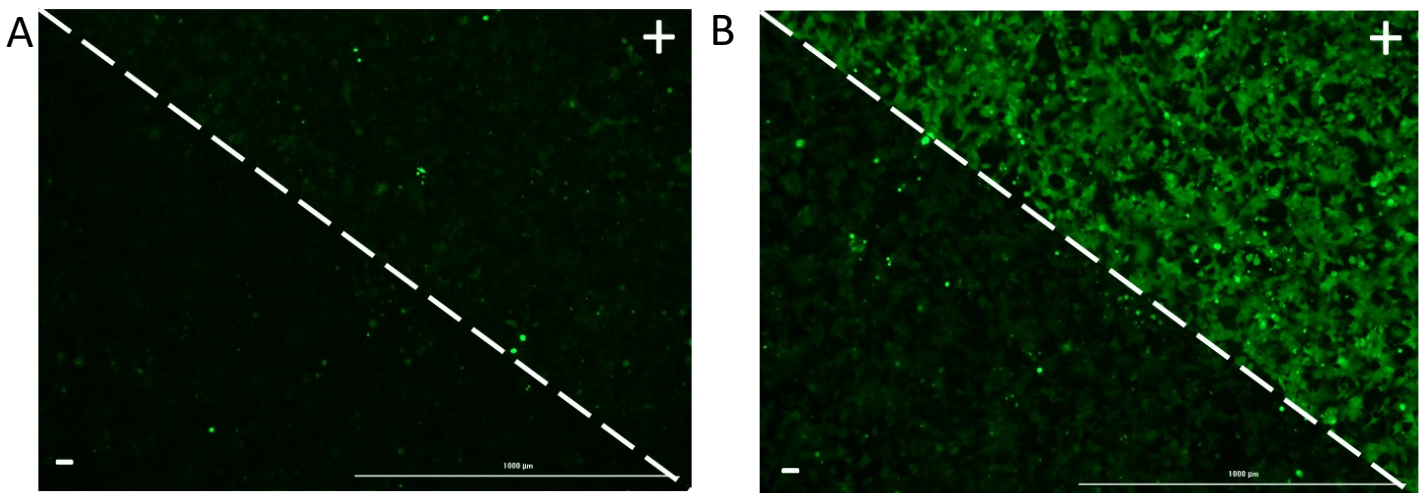
Example Results

Figure 1. Targeting a HEK cell line stably expressing HaloTag® (HT) with ION Thallos-HTL. (A) HEK WT cells were imaged before (-) and after (+) the addition of 1mM TI⁺. **(B)** Monoclonal HEK HT (+) cells were imaged before (-) and after (+) the addition of 1mM TI⁺. The “Dye load, no wash” protocol was used to generate this data. Images were acquired using a Cytation 5.

Example Results

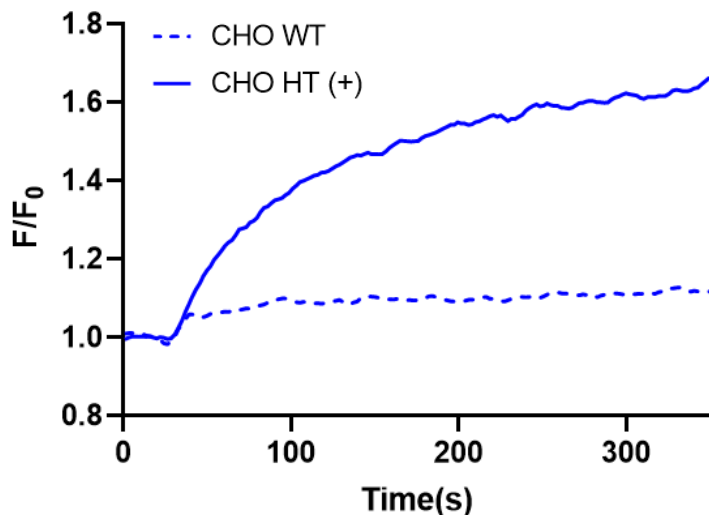


Figure 2. CHO cell response to addition of Ti^+ in no wash HTS assay. CHO K1 cells expressing HaloTag® protein (CHO HT (+)) and CHO K1 cells not expressing the protein (CHO WT) were plated in separate wells in a 96-well plate and loaded with ION Thallos-HTL dye load for one hour ("Dye load, no wash" protocol). Plate was placed in a FlexStation, baseline fluorescence was collected for 30 seconds, stimulus solution containing 1 mM Ti^+ was added and fluorescence data was collected for another 5 minutes.

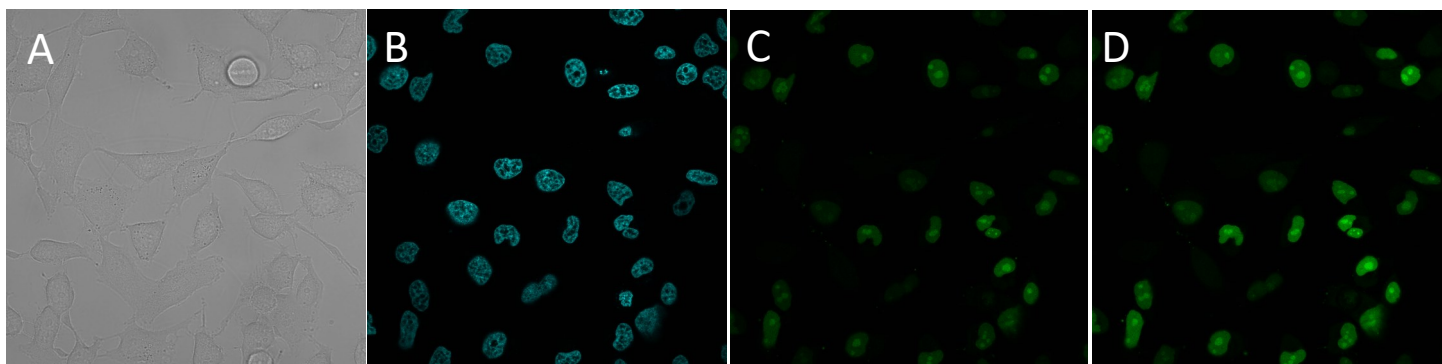


Figure 3. Intracellular localization in HeLa cells. HeLa cells transiently expressing HaloTag® in the nucleus were loaded with ION Thallos-HTL for 30 minutes. Cells were then washed and extra dye was effluxed for 45 minutes prior to imaging ("Dye load with wash" protocol). (B) Hoechst staining was used to stain nuclei to confirm ION Thallos-HTL colocalization. Images were taken (C) before and (D) after addition of Ti^+ (1.67mM). Images were acquired using an Olympus FV3000 Laser Scanning Confocal Microscope and 60X objective.

References

- Macias-Contreras, Miguel et al. "[ION Thallos-HTL: a fluorescent thallium indicator that enables cell-selective and localizable thallium flux assays.](#)" *Organic & biomolecular chemistry* vol. 22,23 4748-4756. 12 Jun. 2024.

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