ION BIOSCIENCES

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Instructions

# ION Thallos Gold-HTL

## Materials Needed

| Name | Volume | Containers | Storage |
|---|---|---|---|
| ION Thallos Gold-HTL AM (25µg vial) | Dry | 1 | -20° C |
| DMSO | 25µL | 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 | 100 µL | 1 | 4° C |
| 100X TRS solution | 100 µL | 1 | 4° C |

## Description

Thallos Gold is a gold fluorescent, intracellular thallium ($Tl^+$) indicator. Thallium indicators have been the gold standard for fluorescence-based potassium ($K^+$) channel HTS for nearly 2 decades. **ION Thallos Gold-HTL** offers the opportunity to localize the indicator to individual cells within a mixed cell population or intracellular compartments using HaloTag® technology, without compromising its $Tl^+$ sensitivity. Once **ION Thallos Gold-HTL** is loaded into cells expressing HaloTag® protein, the indicator covalently binds to the protein. Addition of stimulus solution will show ion flux within the cell type or intracellular compartment of interest.

## 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.

### Dye Load With Efflux Protocol (Recommended for CHO 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.
4. 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]

## Laboratory Procedures (continued)

5. 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 **ION Thallos Gold-HTL** AM in 25 µL of DMSO. After adding DMSO, vortex tube briefly to dissolve the indicator dye, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of indicator 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 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.
10. Incubate in a cell culture incubator at 37ºC for 60 minutes.
11. Make the wash solution with 10 mL of assay buffer and 100 uL of 100X TRS solution. **Do NOT add Probenecid to the efflux solution.
12. After the 60 minute incubation period, wash wells, remove the dye loading solution and add wash solution
13. Incubate cells with wash solution at 37ºC for 45 minutes to efflux the unbound dye
14. Read fluorescence using a plate reader (Ex/Em: 530 nm/550 nm) or image using a fluorescence microscope (using filters for RFP).

### Dye Load Without Efflux 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 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.
5. Vortex conical tube briefly to mix.
6. Dissolve **ION Thallos Gold-HTL AM** in 25 µL of DMSO. After adding DMSO, vortex tube briefly to dissolve the indicator dye, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of indicator dye tube to assay buffer solution to make a dye loading solution.[2]
7. Vortex dye loading solution briefly to mix.
8. 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 µL; 384-well plate, 20 µL.
9. Incubate in a cell culture incubator at 37ºC for 90 minutes.
10. Make the wash solution with 10 mL of assay buffer and 100 uL of 100X TRS solution. **Do NOT add Probenecid to the efflux solution.

## Laboratory Procedures (continued)

11. Read fluorescence using a plate reader (Ex/Em: 530 nm/550 nm) or image using a fluorescence microscope (using filters for RFP).

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

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

## Example Results

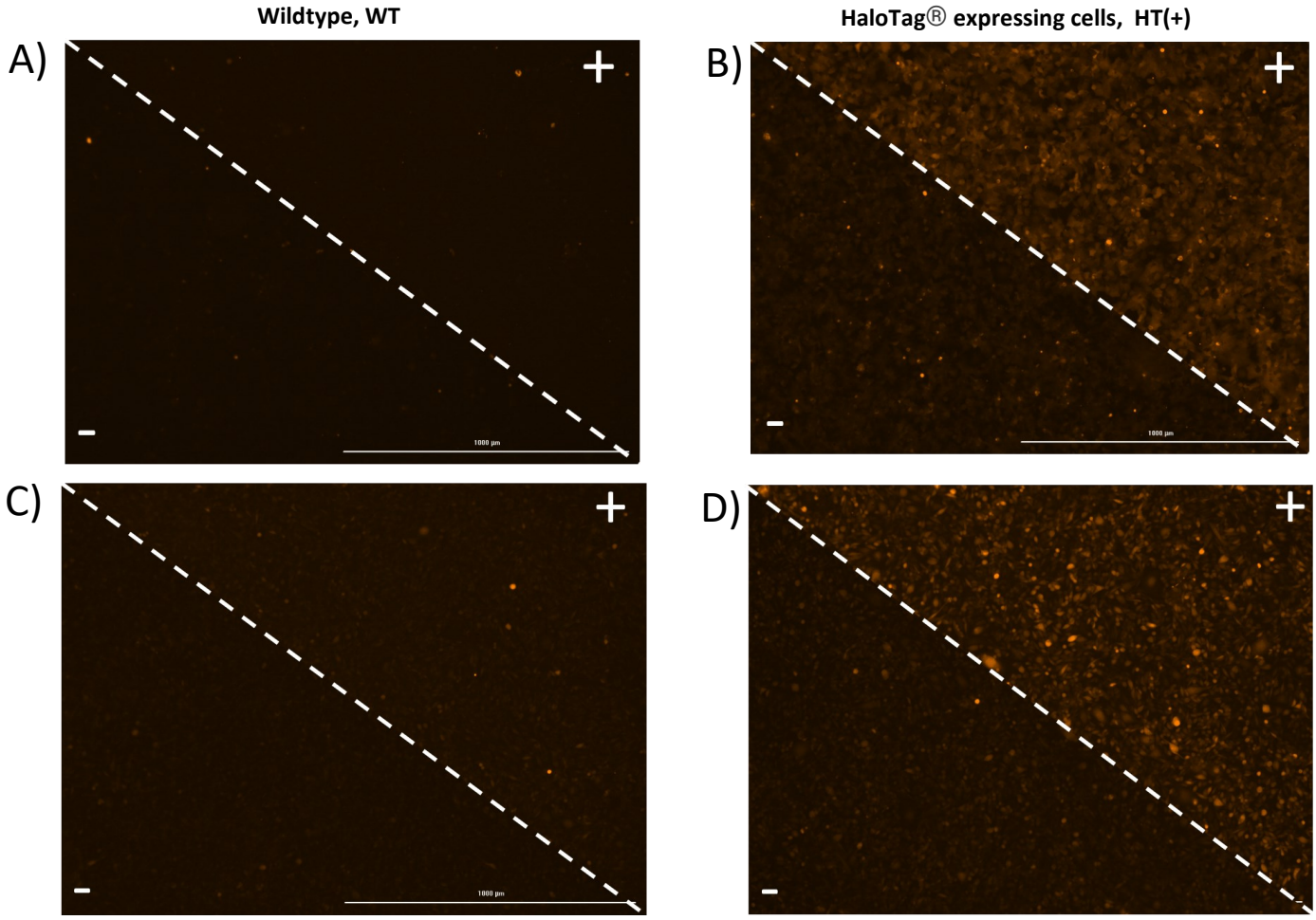


**Figure 1. Targeting HT-expressing HEK and CHO cells with ION Thallos Gold-HTL. (A)** HEK WT and (**C**) CHO WT cells were imaged before (-) and after(+) the addition of 1.7mM $Tl^+$. (**B**) HEK HT (+) and (**D**) CHO HT (+) cells were imaged before (-) and after(+) the addition of 1.7mM $Tl^+$.

## Example Results

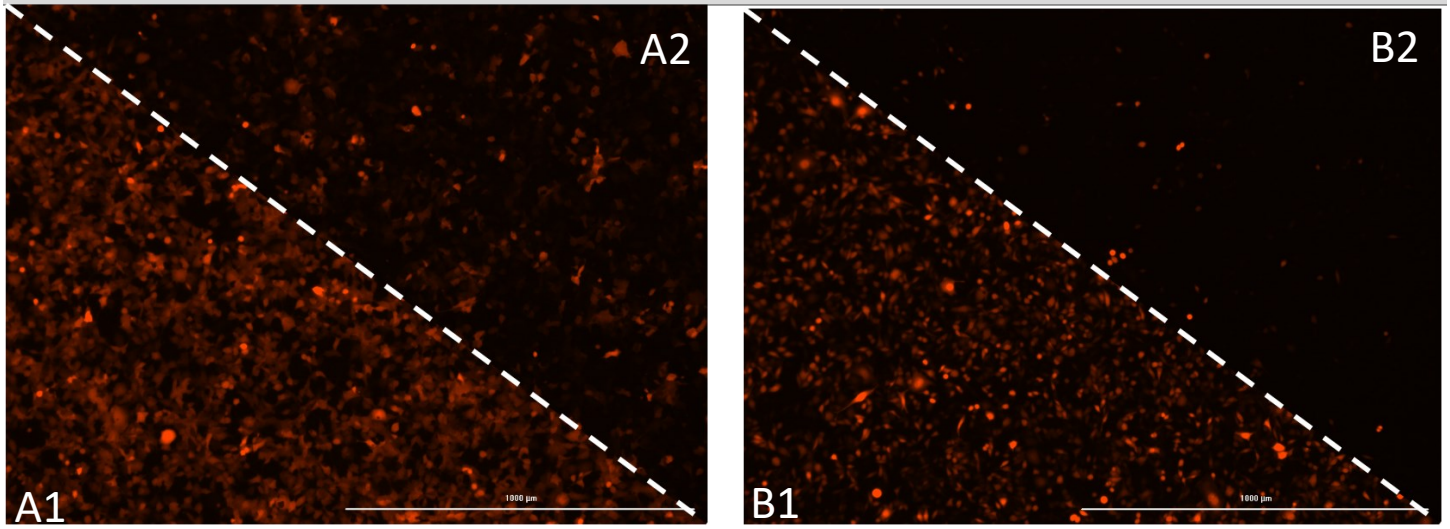


**Figure 2. HEK HT (+) and CHO HT (+) cells loaded with JF585 HaloTag® Ligand .** (**A1**) HEK HT (+) and (**B1**)CHO HT (+) cells were loaded with JF585 HaloTag® Ligand to demonstrate fluorescence when 100% of available HaloTag® protein is bound by the ligand. (**A2**) HEK HT(+) and (**B2**) CHO HT(+) cells were loaded with ION Thallos Gold-HTL first, before JF585 HaloTag® Ligand was added. There is a 70% decrease in fluorescence between images **A1** and **A2**, suggests 70% of the HaloTag protein were bound with ION Thallos Gold-HTL, which is not accessible to JF585 HaloTag® Ligand. There is a 97% decrease in fluorescence between images **B1** and **B2**, suggests 97% of the HaloTag protein were bound with ION Thallos Gold-HTL, which is not accessible to JF585 HaloTag® Ligand.

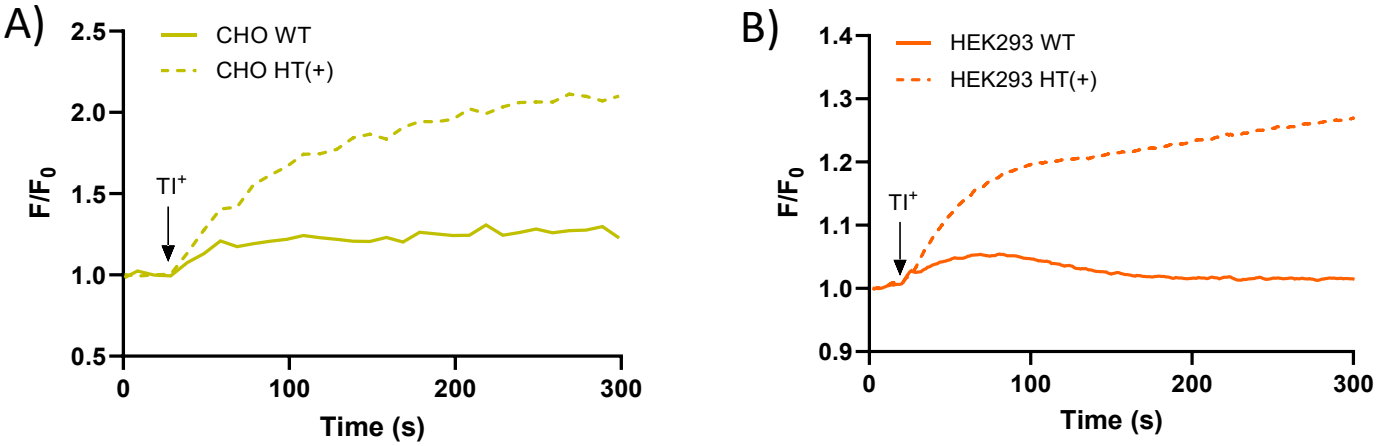


**Figure 3. CHO and HEK cell response to addition of Tl+ in HTS assay. (A)** CHO K1 cells expressing HaloTag® protein (CHO HT (+)) and CHO K1 cells not expressing the protein (CHO WT) were plated in a 96-well plate and Thallos Gold HTL was loaded according to the recommended protocol. Assay readout were performed on readout on Flexstation. Increase of fluorescence was detected only in the CHO HT(+) cells upon addition of stimulus solution containing 1.7mM $Tl^+$. **(B)** HEK293 cells expressing HaloTag® protein (HEK293 HT (+)) and HEK293 cells not expressing the protein (HEK293 WT) were plated in a 384-well plate and Thallos Gold HTL was loaded according to the recommended protocol. Assay readout were performed on readout on Panoptic. Increase of fluorescence was detected only in the CHO HT(+) cells upon addition of stimulus solution containing 1.7mM $Tl^+$.

## Example Results

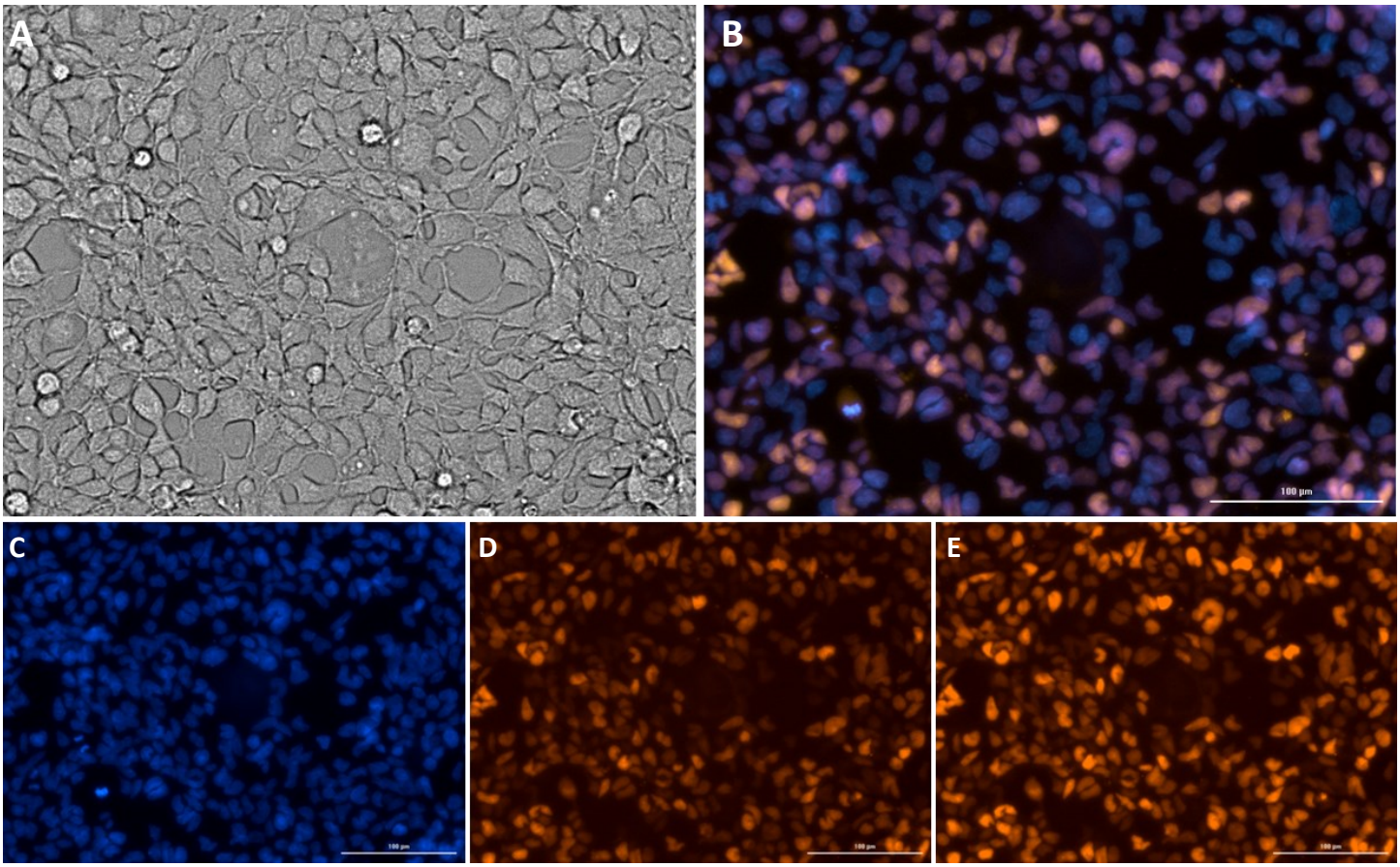


**Figure 4. Nuclear localization of HaloTag® in HEK cells.** HEK cells expressing HaloTag® protein in the nucleus were plated in a 96-well plate and loaded with ION Thallos Gold-HTL dye load for 90 minutes. Dye load was replaced with Hoechst containing assay buffer to stain the nucleus. Wells were loaded for 15 minutes. Buffer was replaced with fresh buffer. (**A**) Brightfield image of HEK cells was collected using Cytation 5. (**B**) Hoechst stain and Thallos Gold-HTL images were merged to depict overlap of fluorescence in both channels. (**C**)DAPI image of Hoechst fluorescence. (**D**) Before and (**E**) after addition of Thallium.