ION Thallos-HaloTag® Ligand (HTL)

Table 1 - Materials Needed	Most Items Available from ION Biosciences	
Name	**Volume**	**Description/Purpose**
ION Thallos-HTL (25 µg vial)	Dry - 1 Vial	Membrane Permeable Thallium Dye Indicator
DMSO[1]	25 µL	Solvent for Dissolution of Dye
100X Pluronic F-127 solution[2]	100 µL	Biocompatible Surfactant for Dye Loading
100X Probenecid solution (optional)[3]	100 µL	Intracellular Dye Retention Aid
50X TRS (optional)[4]	200 µL	Extracellular Fluorescence Masking Agent
Assay Buffer - We Suggest One of the Following:		
⇒ 1X HEPES-Buffered Hank's Balanced Salt Solution	10 mL	1X - Ready to Use Assay Buffer
⇒ 10X Brilliant Thallium Assay Buffer	1 mL	10X - Concentrated Assay Buffer
10X Brilliant Chloride-Free Stimulus Buffer	0.5 - 1 mL	Stimulus Component $[Na^+]$=1.38 M $[K^+]$=0 M
10X High-Potassium, Chloride-Free Stimulus Buffer	0.5 mL	Stimulus Component $[Na^+]$=0 M $[K^+]$=1.38 M
50 mM Thallium Sulfate (Tl_2SO_4) Solution	0.5 mL	Stimulus Component - Thallium Ion (Tl^+)
Water	As Needed	Dilution of 10X Buffer (if used) and Stimulus

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.

General Considerations

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.

Laboratory Procedures - Dye Load with Wash Protocol - Recommended for CHO and HeLa Cells

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 25 µL DMSO[1] to the tube containing ION Thallos-HTL.
3. Vortex until ION Thallos-HTL is fully dissolved. Centrifuge briefly to collect all contents at the tube bottom.
4. Add the appropriate volume (see **Table 2**, below) of 1X Assay Buffer to a 15 mL conical tube. If using 10X Assay Buffer, add the appropriate volume of Water (**Table 3**, below) and 1 mL of 10X Assay Buffer to a 15 mL conical tube.
5. Add 100 µL of 100X Pluronic F-127[2] solution to the conical tube from **step 4**.
6. (Optional) Add 100 µL of 100X Probenecid[3] solution to the conical tube from **step 5**.
7. (Optional) Add 200 µL of 50X TRS[4] solution to the conical tube from **step 6**.
8. Vortex conical tube from **step 7** briefly to mix.
9. Add the entire contents of the ION Thallos-HTL in DMSO solution from **step 3** to the conical tube from **step 8** to make the **Dye Loading Solution**[5].
10. Vortex the **Dye Loading Solution**[5] from **step 9** briefly to mix.

Table 2 Dye Loading Solution[5]	Using 1X Assay Buffer			
Name	**Method A**	**Method B**	**Method C**	**Method D**
ION Thallos-HTL in DMSO[1] Solution	25 µL	25 µL	25 µL	25 µL
100X Pluronic F-127[2] solution	100 µL	100 µL	100 µL	100 µL
100X Probenecid[3] solution (optional)	100 µL	100 µL	-	-
50X TRS[4] (optional)	200 µL	-	200 µL	-
1X HEPES-Buffered Hanks Balanced Salt Solution	9.6 mL	9.8 mL	9.7 mL	9.9 mL
Total	10 mL	10 mL	10 mL	10 mL

Table 3 Dye Loading Solution[5]	Using 10X Assay Buffer			
Name	**Method A**	**Method B**	**Method C**	**Method D**
ION Thallos-HTL in DMSO[1] Solution	25 µL	25 µL	25 µL	25 µL
100X Pluronic F-127[2] solution	100 µL	100 µL	100 µL	100 µL
100X Probenecid[3] solution (optional)	100 µL	100 µL	-	-
50X TRS[4] (optional)	200 µL	-	200 µL	-
10X Brilliant Thallium Assay Buffer	1 mL	1 mL	1 mL	1 mL
Water	8.6 mL	8.8 mL	8.7 mL	8.9 mL
Total	10 mL	10 mL	10 mL	10 mL

Procedure Continues on Next Page

Laboratory Procedures - Dye Load with Wash Protocol (continued)

11. Remove the cell culture medium and add the **Dye Loading Solution**[5] from **step 10**. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.
12. Incubate in a cell culture incubator at 37ºC for 60 minutes.
13. Prepare 10 mL of **Wash Solution** (**Table 4** below) in a 15 mL centrifuge tube by adding the appropriate amounts of assay buffer, water, and 50X TRS if desired. **DO NOT** add Probenecid to the wash solution.

Table 4 Wash Solution

Name	Method A	Method B	Method C	Method D
1X HEPES-Buffered Hanks Balanced Salt Solution	10 mL	9.8 mL	-	-
50X TRS[4] (Optional)	-	200 µL	-	200 µL
10X Brilliant Thallium Assay Buffer	-	-	1 mL	1 mL
Water	-	-	9 mL	8.8 mL
Total	10 mL	10 mL	10 mL	10 mL

14. After the 60 minute incubation period, wash the wells, then incubate at 37ºC for 30 minutes. If TRS is not included in the initial **Wash Solution** in **step 13**, make sure to wash wells again just before proceeding to **step 18**.
15. Prepare a Thallium Stimulus Solution containing ~ 2.5 mM of thallium (Tl^+) ion. **Table 5** below provides two examples of Thallium Stimulus solutions useful for many types of non-voltage-gated and voltage-gated monovalent cation channels and transporters. 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 wells post-thallium stimulus buffer addition should not exceed 4.8 mM due to the solubility limit of thallium in chloride-containing solutions.

Table 5 Thallium Stimulus Solution

Name	Example A	Example B
10X Brilliant Chloride-Free Stimulus Buffer ($[K^+]$ = 0 M, $[Na^+]$ = 1.38 M)	1 mL	0.5 mL
10X High-Potassium, Chloride-Free Stimulus Buffer ($[K^+]$ = 1.38 M, $[Na^+]$ = 0 M)	-	0.5 mL
50 mM Thallium Sulfate (Tl_2SO_4) Solution	0.5 mL	0.5 mL
Water	8.5 mL	8.5 mL
Total	10 mL	10 mL

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Laboratory Procedures - Dye Load with Wash Protocol (continued)

16. Briefly vortex the tube from **step 15** to mix.
17. Add 20 µL per well of the **Thallium Stimulus Solution** from **step 17** to an empty 384-well microplate or 100 µL per well to an empty 96 well plate.
18. Transfer the washed, dye-loaded, cell-containing microplate from **step 14** and the Thallium Stimulus Solution microplate from **step 17** to an imaging fluorescence microscope[6] (using filters for fluorescein or GFP) or a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR, Molecular Devices FlexStation, or BioTek Cytation 5). See **Table 6** for recommended instrument settings.
19. 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 (if using a 384 well plate, or other appropriate volume if using other plate formats) of the Thallium Stimulus Solution to the cell-containing plate and continue data acquisition for an additional 90 seconds[7].

Table 6 Recommended Instrument Settings

Setting	Recommendation
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths[7]	~490 nm/520 nm
Cutoff wavelength	515 nm
Filter selection	GFP, FITC
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing to your application.	

Laboratory Procedures - Dye Load with No-Wash Protocol - Recommended for HEK293 Cells

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 25 µL DMSO[1] to the tube containing ION Thallos-HTL.
3. Vortex until ION Thallos-HTL is fully dissolved. Centrifuge briefly to collect all contents at the tube bottom.
4. Add the appropriate volume (see **Table 7**, next page) of 1X Assay Buffer to a 15 mL conical tube. If using 10X Assay Buffer, add the appropriate volume of Water (**Table 8**, next page) and 1 mL of 10X Assay Buffer to a 15 mL conical tube.
5. Add 200 µL of 100X Pluronic F-127[2] solution to the conical tube from **step 4**.
6. (Optional) Add 200 µL of 100X Probenecid[3] solution to the conical tube from **step 5**.
7. (Optional) Add 400 µL of 50X TRS[4] solution to the conical tube from **step 6**.
8. Vortex conical tube from **step 7** briefly to mix.

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Laboratory Procedures - Dye Load with No-Wash Protocol (continued)

9. Add the entire contents of the ION Thallos-HTL in DMSO solution from **step 3** to the conical tube from **step 8** to make the **Dye Loading Solution**[5].
10. Vortex the **Dye Loading Solution**[5] from **step 9** briefly to mix.

Table 7 Dye Loading Solution[5]	Using 1X Assay Buffer			
Name	**Method A**	**Method B**	**Method C**	**Method D**
ION Thallos-HTL in DMSO[1] Solution	25 µL	25 µL	25 µL	25 µL
100X Pluronic F-127[2] solution	200 µL	200 µL	200 µL	200 µL
100X Probenecid[3] solution (optional)	200 µL	200 µL	-	-
50X TRS[4] (optional)	400 µL	-	400 µL	-
1X HEPES-Buffered Hanks Balanced Salt Solution	9.2 mL	9.6 mL	9.4 mL	9.8 mL
Total	10 mL	10 mL	10 mL	10 mL

Table 8 Dye Loading Solution[5]	Using 10X Assay Buffer			
Name	**Method A**	**Method B**	**Method C**	**Method D**
ION Thallos-HTL in DMSO[1] Solution	25 µL	25 µL	25 µL	25 µL
100X Pluronic F-127[2] solution	200 µL	200 µL	200 µL	200 µL
100X Probenecid[3] solution (optional)	200 µL	200 µL	-	-
50X TRS[4] (optional)	400 µL	-	400 µL	-
10X Brilliant Thallium Assay Buffer	1 mL	1 mL	1 mL	1 mL
Water	8.2 mL	8.6 mL	8.4 mL	8.8 mL
Total	10 mL	10 mL	10 mL	10 mL

11. Remove the cell culture medium and add the **Dye Loading Solution**[5] from **step 10**. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.
12. Incubate in a cell culture incubator at 37°C for 1 - 2 hours.
13. Prepare a Thallium Stimulus Solution containing ~ 2.5 mM of thallium (Tl^+) ion. **Table 5** (next page) provides two examples of Thallium Stimulus solutions useful for many types of non-voltage-gated and voltage-gated monovalent cation channels and transporters. 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 wells post-thallium stimulus buffer addition should not exceed 4.8 mM due to the solubility limit of thallium in chloride-containing solutions.

Procedure Continues on Next Page

Laboratory Procedures - Dye Load with No-Wash Protocol (continued)

Table 9 Thallium Stimulus Solution

Name	Example A	Example B
10X Brilliant Chloride-Free Stimulus Buffer ($[K^+]$ = 0 M, $[Na^+]$ = 1.38 M)	1 mL	0.5 mL
10X High-Potassium, Chloride-Free Stimulus Buffer ($[K^+]$ = 1.38 M, $[Na^+]$ = 0 M)	-	0.5 mL
50 mM Thallium Sulfate (Tl_2SO_4) Solution	0.5 mL	0.5 mL
Water	8.5 mL	8.5 mL
Total	10 mL	10 mL

14. Briefly vortex the tube from **step 13** to mix.
15. Add 20 µL per well of the **Thallium Stimulus Solution** from **step 14** to an empty 384-well microplate or 100 µL per well to an empty 96 well plate.
16. Transfer the dye-loaded, cell-containing microplate from **step 12** and the Thallium Stimulus Solution microplate from **step 15** to an imaging fluorescence microscope[6] (using filters for fluorescein or GFP) or a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR, Molecular Devices FlexStation, or BioTek Cytation 5). See **Table 10** for recommended instrument settings.
17. 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 (if using a 384 well plate, or other appropriate volume if using other plate formats) of the Thallium Stimulus Solution to the cell-containing plate and continue data acquisition for an additional 90 seconds[7].

Table 10 Recommended Instrument Settings

Setting	Recommendation
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths[7]	~490 nm/520 nm
Cutoff wavelength	515 nm
Filter selection	GFP, FITC
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing to your application.	

Example Results

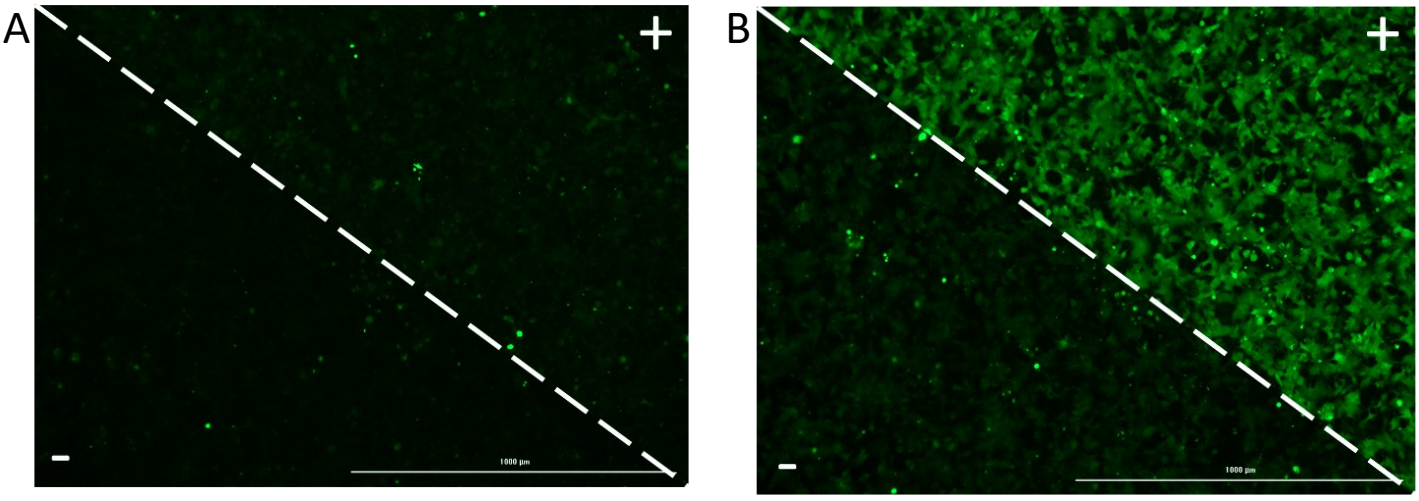

Figure 1. Targeting a HEK cell line stably expressing HaloTag® (HT) with ION Thallos-HTL. (A) HEK WT cells were imaged before (-) and after (+) the addition of 1mM Tl^{+}. (B) Monoclonal HEK HT (+) cells were imaged before (-) and after (+) the addition of 1mM Tl^{+}. The "Dye load, no wash" protocol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

Laboratory Procedures - Footnotes

1 DMSO is hygroscopic and should be stored tightly closed. Wet solvent causes difficulties with dissolution of the dye.

2 Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.

3 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.

4 TRS is a membrane impermeant dye useful for masking extracellular fluorescence. Caution is advised when using TRS or any other extracellular masking solutions as they may have undesirable effects on assay performance for the target of interest.

5 The Dye Loading Solution should be used within 2 hours of dye addition for best results.

6 To minimize extracellular background, the dye loading solution can be replaced with assay buffer containing 1X TRS solution.

7 To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths.

Additional Information

Dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department. Custom and bulk sizes are also available. Contact Sales for more information.

Table 4	Available Reagents	Available Sizes	
Name		Size	Catalog #
ION Thallos-HTL		250 µg x 1 Vial 25 µg x 10 Vials 25 µg x 3 Vials	1381-HTL-C 1381-HTL-F 1381-HTL-G
100X Pluronic F-127 Solution		10 mL Bottle	7601A
100X Probenecid Solution		10 mL Bottle	7300P-100
50X TRS Solution		20 mL Bottle	7060A
1X HEPES-Buffered Hank's Balanced Salt Solution (1X HHBSS)		100 mL Bottle	7001
10X Brilliant Thallium Assay Buffer		10 mL Bottle	7010T
10X Brilliant Chloride-Free Stimulus Buffer		10 mL Bottle	7020B
10X High-Potassium, Chloride Free Stimulus Buffer		10 mL Bottle	7030S
50 mM Thallium Sulfate Solution		5 mL Bottle	7040S