6-Tetramethylrhodamine HaloTag® Ligand (6-TMR-HTL)

Table 1 - Materials		
Name	**Volume**	**Description/Purpose**
6-TMR-HTL (5 mg vial)	Dry - 1 Vial	Membrane Permeable Fluorescent Label
Materials Needed, Not Included		
DMSO[1]	1 mL	Solvent for Dissolution of Dye
Culture Medium	As Needed	Dye-Loading and Washing Cells
Culture Medium without Phenol Red (Optional)	As Needed	Cell-Imaging
1X PBS (Optional)	As Needed	Washing Cells

Description

6-TMR **H**alo**T**ag® **L**igand (6-TMR-**HTL**) is a cell-permeable fluorescent labeling reagent designed for the covalent labeling of HaloTag fusion proteins in live or fixed cells. The bright 6-tetramethylrhodamine (6-TMR, 6-TAMRA) fluorophore enables sensitive detection of HaloTag®-tagged proteins. Upon binding, the ligand forms a stable covalent bond with the Halo-Tag® enzyme, providing long-term signal retention and low background fluorescence. 6-tetramethylrhodamine is conjugated to the HaloTag® chloroalkane ligand through a PEG-2 linker.

6-TMR HaloTag® Ligand is ideal for live-cell imaging, protein localization studies, receptor internalization assays, protein trafficking analysis, HaloTag reporter assays, fluorescence microscopy, confocal imaging, flow cytometry, and high-content screening. ***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 label HaloTag expressing proteins in live cells. All loading conditions (dye concentration, temperature, and time) should be optimized for your specific assay, cell type, and application. ***This dye requires the use of cells transiently or stably expressing HaloTag to work.**

Laboratory Procedures

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 786 µL of DMSO into the tube containing 6-TMR-HTL to obtain a 10 mM solution. Vortex until the 6-TMR-HTL is fully dissolved. Aliquot solution into tubes and store at –20°C. Use small volume aliquots to reduce the number of freeze-thaw cycles each tube will undergo to maximize shelf life.
3. Add 5 µL of the 10 mM 6-TMR-HTL in DMSO solution to 10 mL of warmed Culture Media (1:2000 dilution) and vortex gently to mix to obtain a **5 µM Dye-Loading Solution**. Prepare the Dye-Loading Solution immediately before use.
4. Remove the cell culture medium from the cells and add the **5 µM Dye Loading Solution** from **step 3**. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.
5. Incubate in a cell culture incubator at 37ºC for 15 minutes.
6. After the 15 minute incubation period (**step 5)**, remove the **5 µM Dye Loading Solution** and wash the cells with several volumes of fresh Culture Media or 1X PBS.
7. Add fresh Culture Media to the cells, optionally use Culture Media without Phenol Red to improve imaging results.
8. Transfer the washed, labeled cell-containing microplate from **step 7** to a fluorescence microscope (using Cy3 or TRITC filters) or plate reader (Ex/Em: 550/580 nm).
9. Cells may be processed for additional applications, including: 1. Pulse-chase experiments for measuring protein turnover. 2. Fixation for immunofluorescence. 3. Cell lysis for protein analyses.

Table 2 Recommended Instrument Settings

Setting	Recommendation
Ex/Em wavelengths	~550 nm/580 nm
Cutoff wavelength (if available)	570 nm
Filter selection	Cy3, TRITC
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing to your application.	