Product Information

ViaTag™ Haloalkane Ligands

See product page for a full list of product names, unit sizes, and catalog numbers.

Storage and Handling

Store at -20°C, protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Working solutions should be prepared fresh on the day of use.

Product Technical Information

See product page for spectral properties and other dye-specific technical information. See our Spectra Viewer to view and download the dye excitation and emission spectra.

Product Description

ViaTag™ Haloalkane Ligands are fluorescent ligands for the HaloTag® self-labeling protein tag. The 34 kDa tag sequence is derived from bacterial haloalkane dehydrogenase, and can be fused to proteins of interest for transgenic expression in cells or organisms. The tag enzyme catalyzes the formation of a covalent adduct with a fluorescent ligand containing a reactive haloalkane group. The result is a stably labeled target protein that can be detected by fluorescence microscopy, flow cytometry, or other fluorescence-based detection methods.

Biotium offers ViaTag™ Haloalkane Ligands in both cell membrane permeant and impermeant variations. Cell membrane-permeant haloalkane ligands can be used for labeling tagged intracellular targets in live cells, while cell membrane-impermeant ligands can be used for labeling targets on the cell surface. Because the labeling is covalent it can withstand fixation, permeabilization, or protein extraction after reacting with ligand. In addition, the tag enzyme retains activity after paraformaldehyde fixation, and can be labeled with ligand after fixation and permeabilization of the expressing cells.

Biotium offers a selection of cell membrane-permeant and impermeant ViaTag™ Haloalkane Ligands with a selection of fluorescent dye colors. ViaTag™ Haloalkane Ligands are provided as stable, ready-to-use stock solutions in DMSO. Labeling is rapid, producing bright signal with low background without the need for extensive washing that is required for some ligands.

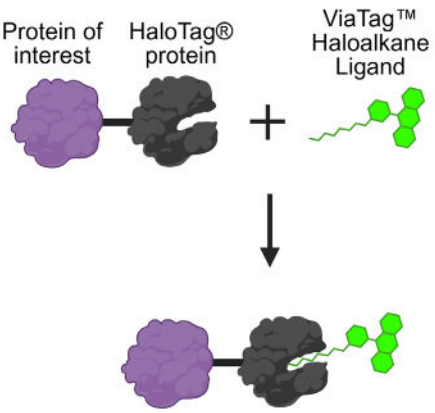

Figure 1. Principle of labeling HaloTag® protein tags with ViaTag™ Haloalkane Ligands. A protein of interest (purple) fused to the HaloTag® fusion protein (black) is specifically labeled with a ViaTag™ Haloalkane Ligand (green) by a covalent bond between the ViaTag™ Ligand and the HaloTag® protein. Created in https://BioRender.com

Experimental Protocols

The following procedures are for labeling HaloTag® fusion proteins in transfected cells for fluorescence microscopy. Labeling time, temperature, concentration, and wash steps may require optimization for different cell or sample types.

Live cell staining

Note: Membrane-permeant ligands will react with tags localized inside the cell or on the cell surface. Membrane-impermeant ligands will only react with tags that are exposed on the cell surface.

1. Dilute ViaTag™ Ligand in cell culture medium. We recommend using 1X (1:1000 dilution) ViaTag™ Ligand as a starting point for titration.
2. Remove the culture medium from the cells and add the medium containing ViaTag™ Ligand.
3. Incubate the cells at 37°C for 30 minutes, protected from light.
4. For cell membrane-impermeant ligands, rinse the cells twice with fresh culture medium. This step is optional for cell membrane-permeant ligands.

   Note: We have not found extensive washing to be required, but additional or longer washes at 37°C may be performed to reduce background, if necessary.

5. Image in the appropriate channel for the ligand. ViaTag™ Ligands are named for their excitation and emission wavelengths. See the product page for recommended laser line and detection filter or verify your instrument settings in our Spectra Viewer.
6. Cells may be processed for downstream applications, including:
   a. Continued culture after washing for pulse-chase experiments.
   b. Fixation, permeabilization, and subsequent immunostaining.
   c. Cell lysis for SDS-PAGE or other protein analysis.

Staining after fixation

1. Rinse transfected cells twice with PBS or other buffer. For adherent cells we recommend using HBSS with calcium/magnesium to maintain cell attachment and morphology.
2. Fix cells with 4% paraformaldehyde in PBS for 15 minutes at room temperature.
3. Rinse cells twice with PBS.
4. Optional: For detecting intracellular targets using a membrane-impermeant ligand, permeabilize the cells with 0.1% Triton® X-100 in PBS for 10 minutes at room temperature. Then, rinse cells twice with PBS.
5. Dilute ViaTag™ Ligand in PBS. We recommend using 1X (1:1000 dilution) ViaTag™ Ligand as a starting point for titration.
6. Remove the buffer from the cells and add the PBS containing ViaTag™ Ligand.
7. Incubate the cells at 37°C for 30 minutes, protected from light.
8. Rinse the cells twice with PBS.

   Note: We have not found extensive washing to be required, but additional or longer washes at 37°C may be performed to reduce background, if necessary.
9. Optional: Cells may be stained for other targets.
10. Image in PBS or fluorescence antifade mounting medium in the appropriate channel for the ligand. ViaTag™ Ligands are named for their excitation and emission wavelengths. See the product page for recommended laser line and detection filter or verify your instrument settings in our Spectra Viewer.

Troubleshooting

Problem	Potential Solutions
No signal or weak signal	• Make sure you are using the appropriate detection settings for the dye. ViaTag™ Ligands are named for their excitation and emission settings. See the product page for recommended filter sets. Note that fluorescence emission in far-red wavelengths (>650 nm) is not visible to the human eye. • For labeling intracellular targets in live cells, make sure you are using a cell membrane-permeant ligand. Intracellular targets also can be labeled after paraformaldehyde fixation and permeabilization. • For weak signal, titrate the ligand at higher concentrations (for example, 1:500 for 2X final concentration, or 1:250 for 4X final concentration). • Confirm the sequence of your fusion protein construct and optimize transfection conditions.
Nonspecific staining or high background	• If signal is bright, titrate the ligand at lower concentrations. For example, dilute 1:2000 for 0.5X ligand concentration, or 1:4000 for 0.25X ligand concentration. • Increase the time and number of wash steps. For example, wash 3 x 5 minutes at 37°C. • For staining of fixed cells, you may include 0.1% Triton® X-100 in wash buffers to reduce background.

Related Products

Cat. No.	Product
92350-92364	Mix-n-Stain™ CF® Dye Small Ligand Labeling Kits
91056	TMP-PEG3-Amine, TFA salt
40081, 40082	NucSpot® Live Nuclear Stains
30105...30142	CellBrite® Steady Membrane Staining Kits
41033-41038	NucSpot® Nuclear Stains
30131...30140	CytoLiner™ Fixed Cell Membrane Stains
00095-00101	ActinBrite™ High Affinity Phalloidin Conjugates
23001, 23002	EverBrite™ Mounting Media, with or without DAPI
23003...23016	EverBrite™ Hardset Mounting Medium, with or without DAPI or NucSpot® 640
70054…70075	MitoView™ Mitochondrial Dyes
70082	MitoView™ Fix 640
70058…70086	LysoView™ Dyes
70065, 70069	LipidSpot™ Lipid Droplet Stains
70062-70064	ViaFluor® Live Cell Microtubule Stains
00081-00087	Transferrin (Human) CF® Dye Conjugates
00068…29127	Cholera Toxin Subunit B CF® Dye Conjugates
80110...80121	CF® Dye Dextran 10,000 MW, Anionic and Fixable
30129…30137	ExoBrite™ True EV Membrane Stains
30127	ExoBrite™ EV Surface Stain Sampler Kit, Green
P028-P029	ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktails

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates and bioconjugates, reactive dyes, Mix-n-Stain™ labeling kits, and other fluorescent probes for cell biology research.