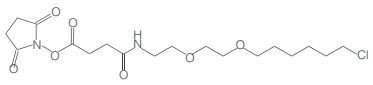
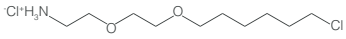
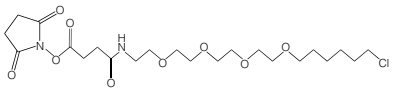
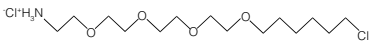
HaloTag® Ligand Building Blocks

Instructions for Use of Products P1691, P6711, P6751 and P6741.

Quick Protocol

Customizable Reactive Ligands Using HaloTag® Ligand Building Blocks

The HaloTag® Ligand Building Blocks[a,b,c] enable a variety of dyes, reporter proteins or nucleic acids with appropriate functional groups to chemically modified with a HaloTag®-reactive chloroalkane moiety.

Product	Size	Cat.#	Structure
HaloTag® Succinimidyl Ester (O2) Ligand	5mg	P1691	
HaloTag® Amine (O2) Ligand	5mg	P6711	
HaloTag® Succinimidyl Ester (O4) Ligand	5mg	P6751	
HaloTag® Amine (O4) Ligand	5mg	P6741	

Storage Conditions: Store Cat.# P1691 and P6751 at below –65°C under inert atmosphere. Store Cat.# P6711 and P6741 at –30°C to –10°C in an air-tight container.

How to Generate HaloTag® Ligands from Building Blocks

The following protocols serve as a guide for generating HaloTag® ligands using HaloTag® Ligand Building Blocks. Section D describes a specific example of generating a fluorophore-containing ligand.

A. General Protocol for Reporter Group Labeling (conjugation to 2-[(6-chloro-hexyloxy)-ethoxy]-ethylamine)

Materials to Be Supplied by the User

- succinimidyl ester reporter group
- base (triethylamine, diisopropylethylamine, etc.)
- DMF (dimethylformamide)
- instrument and reagents for HPLC or silica gel chromatography

1. Add 1.5 to 3 molar equivalents of (02) or (04) amine to one equivalent of the succinimidyl ester of the reporter group in DMF and treat with a molar excess of tertiary amine base (triethylamine, diisopropylethylamine, etc.). For example, use 1ml of DMF solvent per 5mg HaloTag® building block.
2. Stir reaction for 8–16 hours at room temperature.
3. Purify by preparative scale HPLC or silica gel chromatography, depending on lipophilicity of your construct.

B. Protein Labeling with HaloTag® Amine Ligand

Materials to Be Supplied by the User

- protein to be conjugated
- water, 0.1M MES (pH 4.7–6.0) or 0.1M sodium phosphate (pH 7.3)
- DMSO or DMF
- EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride)
- Sephadex® G-25 (for gel filtration separation of ligated protein)

1. Dissolve the protein to be modified at a concentration of 10mg/ml in either water, 0.1M MES (pH 4.7–6.0) or 0.1M sodium phosphate (pH 7.3).
2. Prepare HaloTag® Amine (02 or 04) Ligand stock solution at 5mg/ml in DMSO or DMF.
3. Take a tenfold-molar-excess-to-protein aliquot of the HaloTag® ligand stock solution and dilute it in buffer (same as what is used in Step 1).
4. Add the aqueous HaloTag® ligand stock solution to the protein solution; the final protein concentration will be 5mg/ml or greater.
5. Add EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) in tenfold molar excess to protein as a 0.1–0.5M aqueous solution and react for 2 hours at room temperature.
6. Purify the ligated protein by gel filtration separation using Sephadex® G-25 in the buffer of choice.

C. Antibody Labeling with HaloTag® Succinimidyl Ester Ligand

Materials to Be Supplied by the User

- antibody to be conjugated
- 0.1M sodium phosphate, 0.15M sodium chloride, 10mM EDTA (pH 7.2; pH 7.0 to 7.6 can be used)
- DMSO or DMF
- Sephadex® G-25 (for gel filtration separation of ligated antibody) or dialysis equipment (e.g. MWCO devices or tubing) to separate labeled antibody from reaction byproducts

1. Dissolve antibody in 0.1M sodium phosphate, 0.15M sodium chloride (pH 7.2) at 1–5mg/ml.
2. Prepare HaloTag® Succinimidyl Ester (02 or 04) Ligand stock solution in 5mg/ml DMSO or DMF.
3. Add 10–40µl of HaloTag® Ligand stock solution per millilter of 1mg/ml antibody solution and incubate at room temperature for 30 minutes.

   Note: A 12-fold molar excess works well to ensure at least one label per antibody. Most protocols for general antibody labeling uses 20-fold excess, but higher concentrations of ligand to antibody will label more amines. Furthermore, excess labels may or may not hinder the antibody-epitope interaction.
4. Purify the ligated antibody by gel filtration using Sephadex® G-25 or by dialysis against 0.1M sodium phosphate, 0.15M sodium chloride, 10mM EDTA (pH 7.2).

D. Specific Example Protocol for Generating an XFD488 NHS Ester HaloTag® Ligand

1. Begin with 5mg of XFD488 NHS ester (7.8×10^{-6} mol; e.g., Alexa Fluor® 488 from Life Technologies).
2. Add a small magnetic stir bar directly to the product vial and dissolve the dye in 0.5ml DMF (stored over molecular sieves to remove excess water).
3. With continuous stirring, add a threefold excess of amino ligand (2.3×10^{-5} mol) followed by one drop of N,N-diisoproplyethylamine (excess).

   Note: Add the amine ligand as a stock solution in either DMF or methylene chloride. It stores well at –78°C in solution.

   If using a dye-succidimidyl ester (SE), you can use 1.5 to 3 equivalents of ligand to dye-SE.
4. Mix for 12 hours and monitor by analytical HPLC (C12 or C18) using 0.1% TFA, aqueous phase and acetonitrile, organic phase.
5. Once the reaction is determined to be complete, dilute the reaction mixture with 1ml of water and inject onto a preparative HPLC column (Varian Microsorb, Cat.# 60-8 C18; 250 × 21.4 mm). Purify the compound using the same mobile phase as in the analytical HPLC in Step 4.