

Certificate of Analysis

HaloTag® Biotin Ligand:

Part No.	Size
G828A	30µl
G828B	15µl

Part# 9PIG828

Revised 10/16



Instructions for use of this product can be found in the HaloTag® Technology: Focus on Imaging Technical Manual #TM260, available online at: www.promega.com/protocols

Description: The HaloTag® Interchangeable Labeling Technology is a novel tool for imaging live or fixed mammalian cells that express the HaloTag® Protein or Protein Fusion, analyzing post-translational modification of labeled fusion proteins, and isolating proteins and protein complexes. The HaloTag® Protein is encoded by the a variety of HaloTag® Vectors, which are designed to allow protein fusions. The HaloTag® Biotin Ligand^(a,b) is a small chemical tag that readily crosses the cell membrane and comprises the HaloTag® Reactive Linker and biotin. The HaloTag® Ligands have shown no detectable toxicity or morphological side effects at the recommended labeling conditions in cell lines tested (HeLa, CHO-K1). The HaloTag® Biotin Ligand may be used as an affinity tag to capture a protein of interest using the strong biotin-streptavidin interaction.

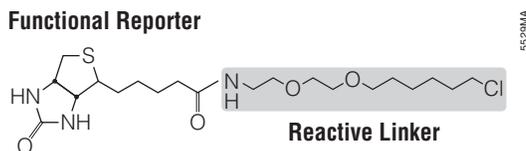
Form: This product is provided as a 5mM ±10% solution in 100% cell-culture quality DMSO.

Formula: C₂₀H₃₆ClN₂O₄S.

Molecular Weight: 450g/mol.

Storage Conditions: See the Product Information Label for storage temperature recommendations and expiration date. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability. We recommend dispensing the ligand into aliquots and storing the aliquots desiccated at -20°C, protected from light.

Structure:



Usage Note: Mix well before use. This ligand is provided in DMSO, which may be harmful to cells at high concentrations. At typical working concentrations, the DMSO is significantly diluted and demonstrates no detectable toxicity or morphological side effects in the cell lines tested (HeLa, CHO-K1).

Quality Control Assays

Identity by H-NMR: Conforms to structure.

Residual Reactive Linker (tested by TLC): ≤5%.

Mass by ES Mass Spectrometry: 450± 2 amu.

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©U.S. Pat. No. RE42931, Japanese Pat. No. 4748685 and other patents pending.

Signed by:

R. Wheeler, Quality Assurance



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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Part# 9PIG828
Printed in USA Revised 10/16.

I. Description

The HaloTag® Interchangeable Labeling Technology is a novel tool for imaging live or fixed cells that express the HaloTag® Protein or protein fusion, analyzing post-translational modification of labeled fusion proteins, or isolating proteins and protein complexes. The technology is based on efficient formation of a covalent bond between a specially designed reporter protein and a specific ligand in living cells, in solution, or on a solid support. The ligand can carry a variety of functionalities, including fluorescent labels, affinity handles and attachments to a solid phase. The covalent bond forms rapidly under physiological conditions and is highly specific and essentially irreversible. The open architecture of the technology enables use of different ligands. We currently offer cell-permeant ligands with red, green and blue fluorophores or biotin.

The HaloTag® Biotin Ligand is cell permeant and can be washed away following incubation with cells. The HaloTag® PEG-Biotin Ligand provides more efficient interaction between the HaloTag® Protein and streptavidin, and thus superior pull-down capabilities. However, it does not cross the cell membrane efficiently and requires that lysates be prepared prior to labeling. The HaloTag® PEG-Biotin Ligand contains a spacer not found in the HaloTag® Biotin Ligand. This provides a significantly longer and more flexible linker between streptavidin and the HaloTag® Protein, which may be advantageous over the HaloTag® Biotin Ligand in preserving the activity of a HaloTag® fusion partner protein upon immobilization or derivatization.

HaloLink™ Resin is a solid support for direct capture of the HaloTag® Protein or protein fusion. Additional ligands will be offered to expand the range of applications.

II. Example Protocol for Capturing and Detecting HaloTag® Protein Expressed in Mammalian Cells

Materials to Be Supplied by the User

- transfection reagent
- endotoxin-free (transfection-grade) plasmid DNA
- cell culture medium
- fetal bovine serum
- streptavidin-coated particles
- 24-well culture plates
- 37°C cell culture incubator
- PBS (37°C)
- Tween® 20
- protease inhibitors (Sigma Cat.# P8340)
- SDS-PAGE sample buffer

This example protocol is intended to serve as a guide. You should empirically optimize the cell culture protocol, transfection conditions, ligand concentration (5–25µM) and labeling protocol for your experimental system. This example protocol was used for CHO-K1 cells (ATCC-CCL61) and performed in 24-well culture plates (Fisher Cat.# 353047).

1. Using standard cell culture and transfection techniques, transfect cells with a plasmid encoding the HaloTag® Protein or Protein Fusion.
2. Prepare a 1,000- to 200-fold dilution of the HaloTag® Biotin Ligand stock solution in 37°C growth medium (5–25µM).
3. Replace growth medium with 200µl/well of the diluted HaloTag® Biotin Ligand stock solution.

4. Incubate cells with the HaloTag® Biotin Ligand for 15 minutes at 37°C, 5% CO₂.
5. Remove the HaloTag® Biotin Ligand-containing medium, and quickly rinse the cells with 1ml/well warm PBS (37°C). Repeat two times for a total of three rinses.
6. Replace the PBS with fresh growth medium (37°C), and return the cells to the incubator for 60 minutes at 37°C, 5% CO₂.
7. Quickly wash the cells 2 times with 1.0ml/well PBS (37°C).
8. Add 200µl PBS containing protease inhibitors (Sigma Cat.# P8340) to each well as recommended by the manufacturer.
9. Lyse the cells by mechanical disruption.
10. Use cell lysates immediately, or store them at –20°C for up to 1 month.

Capturing Biotinylated HaloTag® Protein

11. Perform capture according to the instructions provided by the manufacturer of the streptavidin-coated paramagnetic particles.
You may need to optimize the amount of cell lysate and amount of streptavidin particles for your experimental system.
12. To collect proteins bound to the particles add ~50–60µl of SDS-PAGE sample buffer to the particles, and heat the suspension at 95°C for 5 minutes.
13. Capture magnetic particles.
14. Carefully collect the supernatant (containing the bound proteins).
15. Analyze samples immediately, or store them at –20°C. Proteins can be resolved on SDS-PAGE and analyzed by Western blot.

Note: Capture of some enzymes can be detected directly on the particles with an appropriate enzyme activity assay.

III. Related Products

Product	Size	Cat.#
HaloTag® TMR Ligand	30µl	G8251
	15µl	G8252
HaloTag® diAcFAM Ligand	30µl	G8272
	15µl	G8273
HaloTag® Coumarin Ligand	30µl	G8581
	15µl	G8582
HaloTag® Oregon Green® Ligand	30µl	G2801
	15µl	G2802
HaloTag® PEG-Biotin Ligand	30µl	G8591
	15µl	G8592
HaloLink™ Resin	40 reactions (2ml)	G1911
	100 reactions (5ml)	G1912