Certificate of Analysis

HaloTag® PEG-Biotin Ligand:

Part No.	Size
G859A	30µl
G859B	15µl

Part# 9PIG859

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**

AF9PIG859 1016G859

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 HaloTag® Protein is encoded by a variety of HaloTag® Vectors, which are designed to allow construction of protein fusions. The HaloTag® PEG-Biotin Ligand[a,b] is a chemical tag that consists of a hydrophilic spacer with four ethylene gylcols between the HaloTag® Reactive linker and biotin. This ligand does not cross the cell membrane efficiently and requires that lysates be prepared prior to labeling. The spacer provides additional length to the ligand, resulting in a highly efficient interaction between the HaloTag® Protein and streptavidin. The spacer also provides a flexible linker between the HaloTag® Protein and streptavidin, which may be beneficial to preserving the activity of a HaloTag® fusion partner protein upon immobilization or derivatization.

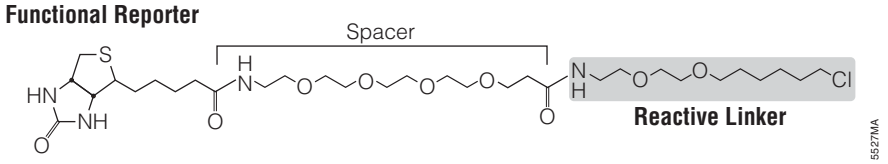
Form: This product is provided as a 5mM ± 10% solution in 100% cell-culture-quality DMSO.

Formula: $C_{32}H_{57}ClN_4O_9S$.

Molecular Weight: 697g/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. The 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 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: 697±2 amu

[a]BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE LABEL LICENSE. If researcher is not willing to accept the terms of this label license, and the product is unused, Promega will accept return of the unused product and provide researcher with a full refund.
Researcher may use this product for research use only; no transfer or commercial use of this product is allowed. Commercial use means any and all uses of this product by a party in exchange for consideration, including, but not limited to (1) use in further product manufacture; (2) use in provision of services, information or data; and (3) resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research. With respect to any uses outside this label license, including any commercial, diagnostic, therapeutic or prophylactic uses, please contact Promega for supply and licensing information. PROMEGA MAKES NO REPRESENTATIONS OR WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED, INCLUDING FOR MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE WITH REGARDS TO THE PRODUCT. The terms of this label license shall be governed under the laws of the State of Wisconsin, USA.

[b]U.S. Pat. No. RE42931, Japanese Pat. No. 4748685 and other patents pending.

Signed by:

R. Wheeler, Quality Assurance

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399 USA
Telephone 608-274-4330
Toll Free 800-356-9526
Fax 608-277-2516
Internet www.promega.com

Part# 9PIG859
Printed in USA. Revised 10/16.

Promega

Usage Information

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 incubations 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
- tissue culture plates
- 37°C cell culture incubator
- 1X PBS (phosphate-buffered saline; cold and 37°C)
- 1X TBS (Tris-buffered saline)
- BSA [Bovine Serum Albumin (Cat.# W3841)]
- Tween® 20 (Cat.# W3831) or IGEPAL® CA-630 (Sigma Cat.# I8896)
- protease inhibitors (Sigma Cat.# P8340 or Roche Biochemicals Cat.# 1-836-153)
- Streptavidin MagneSphere® Paramagnetic Particles (Cat.# Z5481 or Z5482)
- Magnetic Separation Stand (Cat.# Z5331, Z5332, Z5333, Z5341, Z5342, Z5343, or Z5410)
- SDS PAGE sample buffer
- Optional: RQ1 RNase-Free DNase (Cat.# M6101)

This example protocol is intended to serve as a guide. You should empirically optimize the cell culture protocol, transfection conditions, ligand concentration (1–5µM), labeling incubation time (2–4 hours) and capture protocols for your experimental system. This example protocol was used for CHO-K1 cells (ATCC-CCL61) and HeLa cells (ATCC-CCL2) and performed in 100mm dishes, 6-well culture plates, or 24-well culture plates. The volumes indicated are for a 24-well culture plate and can be scaled up accordingly for larger wells or plates.

A. Transfection and Cell Lysis

1. Using standard cell culture and transfection techniques, transfect cells with a plasmid encoding the HaloTag® Protein or Protein Fusion.
2. Allow transfected cells to express the HaloTag® Protein or Protein Fusion for 24–48 hours.
3. Remove growth medium, and rinse the cells with 1ml/well warm 1X PBS (37°C).
4. Add 200µl cold 1X PBS containing protease inhibitors to each well as recommended by the manufacturer.
   Note: DNase may be added to reduce viscosity (1µl RQ1 DNase per 100µl lysate).
5. Scrape the cells from the plastic, and lyse the cells by mechanical disruption (shearing through an 18-gauge needle, Dounce homogenization and/or sonication).
6. The lysate may be centrifuged for 5 minutes at 14,000rpm in a microcentrifuge and the supernatant saved as a cleared lysate.
7. Use cell lysates immediately, or store them at –20°C for up to 1 month.

B. In Vitro Labeling with HaloTag® PEG-Biotin Ligand

1. Incubate 100µl cell lysate in the prescence of HaloTag® PEG-Biotin Ligand for 2–4 hours at room temperature. The final recommended working concentration is 1µM.

C. Capturing Biotinylated HaloTag® Protein

1. Prepare 100µl Strepavidin MagneSphere® Paramagnetic Particles by washing three times with 300µl 1X TBS (i.e., three times the original volume of particles) containing ≤0.05% Tween® 20 or 0.001% IGEPAL® CA-630. Capture particles using a magnetic stand between washes. Allow particles to bind to the magnet for at least 1 minute before removing liquid.
   Note: You will need to optimize the amount of ligand and particles for each experimental system. We recommend using 100µl particles when labeling with 1µM ligand and 500µl particles when labeling with 5µM ligand. This ratio must be maintained to allow sufficient biotin binding sites for unreacted HaloTag® PEG-Biotin Ligand.
   Note: You may need to optimize the type and concentration of detergent for each experimental system.
2. Following the final wash, capture the particles, remove the buffer and add labeled lysate to the particles, pipetting to resuspend and mix.
3. Incubate the lysate with the particles for 30 minutes at room temperature with shaking to allow continuous mixing.
4. Capture particles, and wash five times with 300µl of 1X TBS (i.e., three times the original volume of particles) containing protease inhibitors, 0.5mg/ml BSA, and ≤0.05% Tween®-20 or 0.001% IGEPAL® CA-630.
5. Following the final wash step, capture the particles and resuspend in ~50µl of 1X SDS-PAGE sample buffer, and heat the suspension for 5 minutes at 95°C.
   Note: You may need to optimize the resuspension volume when using more particles. We recommend using 200µl 1X SDS-PAGE sample buffer when resuspending 500µl particles.
6. Capture magnetic particles, and carefully collect the supernatant (containing the bound proteins).
7. 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 proteins can be detected directly on the particles with an appropriate enzyme activity assay.

III. Related Products

Product	Size	Cat.#
HaloTag® diAcFAM Ligand	30µl	G8272
	15µl	G8273
HaloTag® TMR Ligand	30µl	G8251
	15µl	G8252
HaloTag® Coumarin Ligand	30µl	G8581
	15µl	G8582
HaloTag® Oregon Green® Ligand	30µl	G2801
	15µl	G2802
HaloTag® Biotin Ligand	30µl	G8281
	15µl	G8282
HaloLink™ Resin	40 reactions (2ml)	G1911
	100 reactions (5ml)	G1912

Summary of Changes, 9/15 Revision

The following change was made to the 9/15 version of this document:
A legal disclaimer was updated.

Part# 9PIG859
Printed in USA. Revised 10/16.