Certificate of Analysis

HaloTag® Biotin Ligand:

Part# 9PIG828
Revised 5/24

Cat.#	Size
G828A	30µl
G928B	15µl

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

AF9PIG828 0524G828

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.

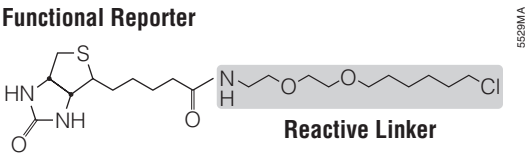
Form: This product is provided as a 5mM ±10% solution in 100% cell-culture quality DMSO.

Formula: $C_{20}H_{36}ClN_3O_4S$.

Molecular Weight: 450g/mol.

Storage Conditions: See the product information label for storage temperature recommendations and expiration date. Avoid multiple freeze-thaw cycles Land 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.

[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

PRODUCT USE LIMITATIONS, WARRANTY, DISCLAIMER

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

Part# 9PIG828
Printed in USA. Revised 5/24.

Promega

Usage Information

1. 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.

2. 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% CO_2.
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% CO_2.
7. Quickly wash the cells two 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 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.

3. 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® R110Direct™ Ligand	30µl	G3221
HaloTag® TMRDirect™ Ligand	30µl	G2991
HaloTag® PEG-Biotin Ligand	30µl	G8591
	15µl	G8592
HaloLink™ Resin	1.25ml	G1912
	2.5ml	G1913
	10ml	G1914
	25ml	G1915
HaloTag® Mammalian Pull-Down System	1 each	G6504
HaloTag® Mammalian Pull-Down and Labeling System	24 reactions	G6500
HaloTag® Complete Pull-Down System	24 reactions	G6509
HaloTag® Control Vector	20µg	G6591

Part# 9PIG828
Printed in USA. Revised 5/24.