Certificate of Analysis

pHTN HaloTag® CMV-neo Vector:

Part No.	Size
G772A	20µg

Part# 9PIG772

Revised 1/18

AF9PIG772 0118G772

Description: The pHTN HaloTag® CMV-neo Vector[a,b] is configured to append the HaloTag® tag to the amino-terminus of the fused protein. The vector provides constitutive high-level protein expression in mammalian cells using the human cytomegalovirus (CMV) immediate early enhancer/promoter. The vector contains a multiple cloning region for convenient cloning, and can be used for both transient and stable gene expression. The stable expression is mediated by co-expression of the neomycin phosphotransferase gene, which confers resistance to the antibiotic G-418 (Cat.# V7983). Alternatively, the HaloTag® coding region and the accompanying linker can be removed (either using PCR or restriction enzyme digestion) and transferred into other commercially available vectors.

Note: The insert must contain an in-frame stop codon for translation termination.

The pHTN HaloTag® CMV-neo Vector contains the following features:

- A CMV immediate-early enhancer/promoter for constitutive expression in mammalian cells.
- A T7 RNA polymerase promoter for in vitro HaloTag® fusion protein expression in cell-free systems (e.g., TNT® lysate reaction).
- A multiple cloning region containing unique restriction sites to facilitate gene insertion into the vector.
- The N-terminal HaloTag® region, which rapidly forms covalent bonds with HaloTag® ligands and surfaces, enabling labeling and immobilization of expressed proteins.
- A HaloTag® linker, a stretch of amino acids that allows efficient flexibility of the HaloTag® tag when fused to the protein of interest.
- A TEV protease site for cleavage of the expressed protein from HaloTag® coding region using TEV protease.
- An ampicillin-resistance gene for selection of plasmid in bacteria.

Concentration: 1µg/µl.

GenBank® Accession Number: JF920304.

Storage Buffer: The pHTN HaloTag® CMV-neo Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 7.4).

Storage Conditions: See Product Information Label for storage recommendations and expiration date. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

Usage Notes:

1. For stable expression, the transfected cells must be selected with the antibiotic G-418. Following transfection, seed the cells at low density, and apply the G-418 antibiotic to the medium at a concentration 100µg/ml–1mg/ml. For effective selection, the cells should be subconfluent; nongrowing cells are resistant to the effects of G-418. The concentration of G-418 required to select and maintain drug resistance depends on the cell type and growth rate. In general, mammalian cells require a concentration of 400–600µg/ml of G-418 for selection and 200–400µg/ml of G-418 for maintenance of stable transfectants. Change the growth medium every 3 days until drug-resistant clones appear (2–5 weeks, depending on the cell type). For cells not expressing neomycin phosphotransferase, cell death should occur 3–9 days after adding G-418.
2. When removing the HaloTag® gene to insert into other vectors, it is critical to also include the HaloTag® linker and the TEV protease recognition sequence to ensure best function of the HaloTag® coding region.

Quality Control Assays

Contaminant Assays

Contaminating Nucleic Acids: RNA, single-stranded DNA and chromosomal DNA are not evident in an overload sample of this vector as determined by agarose gel electrophoresis.

Nuclease Assay: To demonstrate the absence of endonucleases and exonucleases, vector DNA is incubated in standard digest buffers at 37°C for 16 hours followed by agarose gel electrophoresis. The specification is <10% conversion to nicked or linear DNA.

Physical Purity: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Functional Assays

Identity Assay: The vector has been sequenced completely and has 100% identity with the published sequence available at: www.promega.com/products/vectors

Restriction Enzyme Digests: Vector DNA is analyzed for the presence of certain restriction enzyme sites by incubation with a variety of restriction enzymes at the specified digestion temperature for 1 hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

Signed by:

R. Wheeler, Quality Assurance

Promega

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Usage Information

pHTN HaloTag® CMV-neo Vector Features and Circle Map

The following features are present in the pHTN HaloTag® CMV-neo Vector based on nucleotide sequence.

Feature	Position
CMV intermediate early enhancer/promoter	1–742
Chimeric intron	857–989
T7 RNA polymerase promoter (–17 to +3)	1033–1052
HaloTag® coding region	1067–1957
HaloTag® linker	1958–1996
TEV protease recognition sequence	1970–1990
Multiple cloning region	1997–2072
SV40 late polyadenylation signal	2135–2356
SV40 enhancer and early promoter	2455–2873
SV40 minimum origin of replication	2771–2836
Neomycin phophotransferase coding region	2918–3712
Synthetic polyadenylation signal	3776–3824
β-lactamase (Amp^r) coding region	4085–4945
*Col*E1-derived plasmid origin of replication	5100–5136

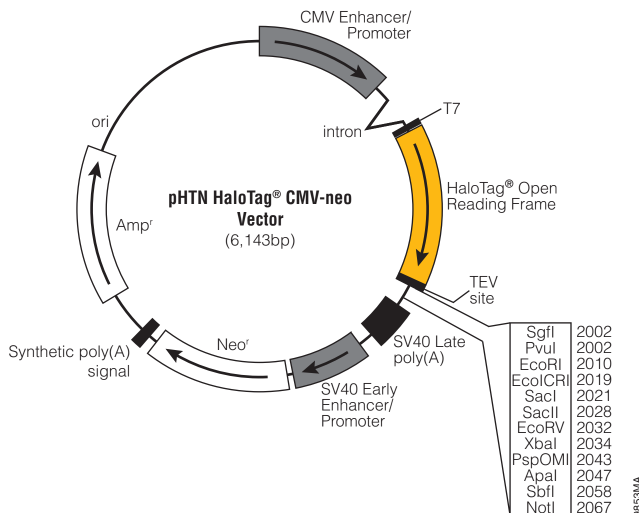

Figure 1. pHTN HaloTag® CMV-neo Vector circle map and sequence reference points.

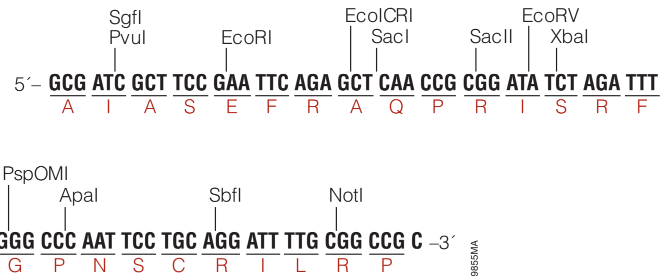

Figure 2. pHTN HaloTag® CMV-neo Vector multiple cloning region sequence and unique restriction sites. The amino acid sequence corresponds to the correct reading frame for the HaloTag® coding region.

Related Products

Product	Size	Cat.#
JM109 Competent Cells, >10⁸cfu/µg	5 × 200µl	L2001
JM109 Competent Cells, >10⁷cfu/µg	5 × 200µl	L1001
HB101 Competent Cells, >10⁸cfu/µg	5 × 200µl	L2011
HaloTag® Mammalian Protein Detection and Purification System	1 each	G6795
HaloTag® Mammalian Pull-Down and Labeling System	24 reactions	G6500
HaloCHIP™ System	20 reactions	G9410

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Researchers may use this product for research use only, no commercial use is allowed. Researchers shall have no right to modify or otherwise create variations of the nucleotide sequence of the HaloTag® gene. Researchers may however clone heterologous DNA sequences at either or both ends of said HaloTag® gene so as to create fused gene sequences provided that the coding sequence of the resulting HaloTag® gene has no more than four (4) deoxynucleotides missing at the affected terminus when compared to the intact HaloTag® gene sequence. In addition, researchers must do one of the following in conjunction with use of the product: (1) use Promega HaloTag® ligands, which can be modified or linked to Promega or customer-supplied moieties, or (2) contact Promega to obtain a license if Promega HaloTag® ligands are not to be used. Researchers may transfer derivatives to others for research use provided that at the time of transfer a copy of this label license is given to the recipients and recipients agree to be bound by the terms of this label license. With respect to any uses outside this label license, including any 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 agreement shall be governed under the laws of the State of Wisconsin, USA.

[b]U.S. Pat. Nos. 7,425,436, 7,935,803, 8,466,269, 8,742,086, 8,420,367 and 8,748,148 and other patents and patents pending.

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