

Certificate of Analysis

pH6HTN His₆HaloTag[®] T7 Vector:

Part No.
G797A

Size
20µg

Description: The pH6HTN His₆HaloTag[®] T7 Vector^(a,b) is configured to append the His₆HaloTag[®] tag to the amino-terminus of the fused protein. The vector contains a multiple cloning region for convenient cloning and a His₆HaloTag[®] protein coding region that allows for both purification and labeling of the expressed fusion protein.

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

The pH6HTN His₆HaloTag[®] T7 Vector contains the following features:

- A **T7 RNA polymerase promoter** for in vitro His₆HaloTag[®] fusion protein expression in cell-free systems (e.g., TnT[®] lysate reaction) and in vivo expression in *E. coli* strains containing T7 RNA polymerase.
- A **multiple cloning region** containing unique restriction sites to facilitate gene insertion into the vector.
- The **N-terminal His₆HaloTag[®] region**, which allows simple purification via the hexahistidine tag and rapid formation of covalent bonds with HaloTag[®] ligands and surfaces, allowing 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 His₆HaloTag[®] coding region using HaloTEV Protease (Cat.# G6601).
- An **ampicillin-resistance gene** for selection of plasmid in bacteria.

Concentration: 1µg/µl.

GenBank[®] Accession Number: JN874646.

Storage Buffer: The pH6HTN His₆HaloTag[®] T7 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 Note: 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.

Part# 9PIG797

Revised 2/17



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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.

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All specifications are subject to change without prior notice.

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.

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Signed by:

R. Wheeler, Quality Assurance

pH6HTN His₆HaloTag® T7 Vector Features and Circle Map

The following features are present based on nucleotide sequence.

T7 RNA polymerase promoter (–17 to +3)	21–40
His ₆ HaloTag® coding region	70–981
His ₆ region	76–93
HaloTag® region	94–981
HaloTag® linker region	982–1020
TEV protease recognition sequence	994–1014
N-terminal multiple cloning region	1021–1096
T7 terminator	1132–1179
β-lactamase (Amp ^r) coding region	1513–2373
Col/E1-derived plasmid origin of replication	2528–2564
<i>rrnB</i> transcription terminator	3571–3972

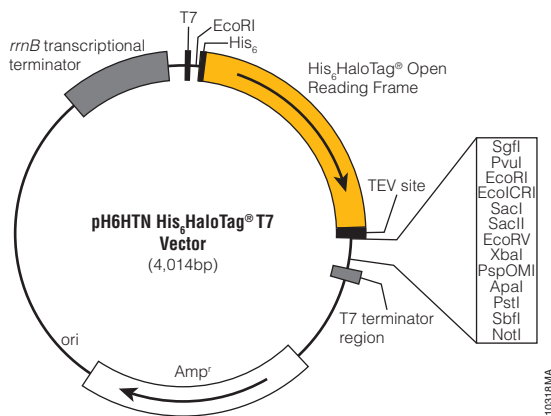


Figure 1. pH6HTN His₆HaloTag® T7 Vector circle map and sequence reference points.

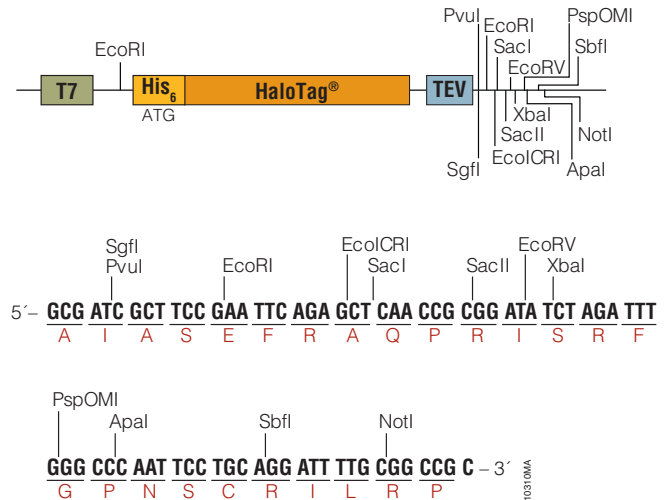


Figure 2. pH6HTN His₆HaloTag® T7 Vector multiple cloning region sequence and unique restriction sites. The amino acid sequence corresponds to the correct reading frame for the HaloTag® coding region.

Related Products

Product	Size	Cat. #
JM109 Competent Cells, >10 ⁸ cfu/μg	5 × 200μl	L2001
JM109 Competent Cells, >10 ⁷ cfu/μg	5 × 200μl	L1001
HB101 Competent Cells, >10 ⁸ cfu/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
HaloTEV Protease	1,000 units	G6601
	4,000 units	G6602

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