

Certificate of Analysis

pFN18K HaloTag® T7 Flexi® Vector:

Part No.	Size
G268A	20µg

Part# 9PIG268

Revised 10/16

Description: The pFN18K HaloTag® T7 Flexi® Vector^(a-d) is configured to append the HaloTag® tag to the amino-terminus of the protein fusion partner and provides T7 RNA polymerase-driven protein expression in *E. coli* or in cell-free translation systems.

The pFN18K HaloTag® T7 Flexi® Vector contains the following features:

- A **T7 RNA polymerase promoter** for in vitro HaloTag® fusion protein expression in cell-free systems (e.g., T7 *E. coli* S30 extract).
- The **N-terminal HaloTag® region**, which rapidly forms covalent bonds with HaloTag® ligands, enabling labeling or immobilization of expressed proteins.
- A **TEV protease site** for cleavage of the expressed protein from HaloTag® using ProTEV Protease (Cat.# V6051).
- The lethal **barnase gene** for positive selection of the insert. **Note: the pFN18K HaloTag® T7 Flexi® Vector can only be propagated in *E. coli* once the barnase gene is replaced with the protein-coding sequence of interest.**
- A **kanamycin-resistance gene** for selection of the plasmid.
- Unique **SgfI and PmeI sites**, which allow easy insertion of the sequence of interest. These sites create a readthrough sequence that can be joined to a protein-coding region flanked by SgfI and PmeI sites, enabling easy transfer to the pFN18K HaloTag® T7 Flexi® Vector from other Flexi® Vectors with different expression options.
- A ***rmb* transcription terminator** for preventing in vivo *E. coli* transcription into the insert.

Concentration: 100ng/µl.

GenBank® Accession Number: EU545993.

Storage Buffer: The pFN18K HaloTag® T7 Flexi® Vector is supplied in 10mM Tris-HCl (pH 8.0), 1mM EDTA.

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

Usage Notes:

1. This vector was designed to be used with the Flexi® Vector System, a directional cloning method to shuttle protein-coding sequences between compatible vectors. To prepare the HaloTag® fusion protein, the protein coding region is cloned into the pFN18K HaloTag® T7 Flexi® Vector using the Flexi® System, Entry/Transfer (Cat.# C8640). For more information, see the *Flexi® Vector Systems Technical Manual #TM254*, available online at: www.promega.com/protocols
2. Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.



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Promega

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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.

Part# 9PIG268
Printed in USA. Revised 10/16.

Quality Control Assays

Contaminant Assays

Contaminating Nucleic Acids: RNA, single-stranded DNA and chromosomal DNA are not evident in specified quantities of the vector as determined by agarose gel electrophoresis.

Nuclease Assay: Following incubation of 1µg of the vector in Restriction Enzyme Buffer at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

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/vectors/

Restriction Digestion: The functional purity of the vector DNA is verified by successful digestion with restriction enzymes at the optimal temperature for one hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

Signed by:

R. Wheeler, Quality Assurance

pFN18K HaloTag® T7 Flexi® Vector Features and Circle Map

The following features are present in the vector based on nucleotide sequence.

T7 RNA polymerase promoter (-17 to +3)	21-40
HaloTag® protein coding region	70-960
TEV site	973-993
Sgfl site	1000-1007
barnase coding region	1031-1366
Pmel site	1368-1375
T7 terminator	1495-1542
Kanamycin resistance (Kan ^r) coding region	1923-2717
<i>Col</i> E1-derived plasmid origin of replication	2886-2922
<i>cer</i> site (site for <i>E. coli</i> XerCD recombinase)	3593-3878
<i>rnnB</i> transcription terminator	3929-4330

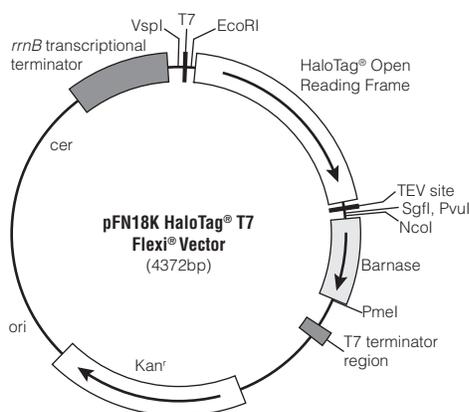


Figure 1. pFN18K HaloTag® T7 Flexi® Vector circle map and sequence reference points.

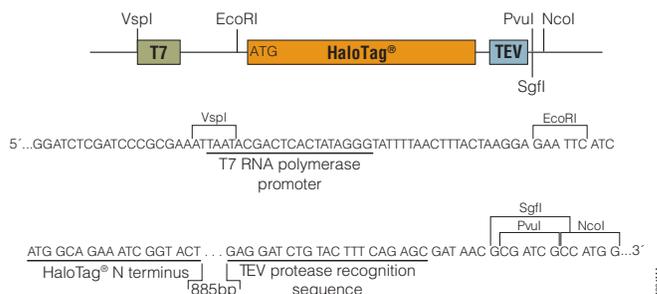


Figure 2. pFN18K HaloTag® T7 Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

Related Products

Product	Size	Cat.#
HaloTag® Cloning Starter System		G6050
Flexi® System, Entry/Transfer	5 entry and 20 transfer reactions	C8640
Flexi® System, Transfer	100 transfer reactions	C8820
Carboxy Flexi® System, Transfer	50 transfer reactions	C9320
10X Flexi® Enzyme Blend (Sgfl & Pmel)	25µl	R1851
	100µl	R1852
Carboxy Flexi Enzyme Blend (Sgfl & EcoRI)	50µl	R1901
Single Step (KRX) Competent Cells	20 x 50µl	L3002

Summary of Changes

The following changes were made to the 12/14 revision of this document:

- Expired patent or license statements were removed.

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Printed in USA. Revised 10/16.