Certificate of Analysis

pFN28K HaloTag® CMV-neo Flexi® Vector:

Part No.	Size
G845A	20µg

Part# 9PIG845

Revised 11/21

Description: The pFN28K HaloTag® CMV-neo Flexi Vector[a–d] is configured to append the HaloTag® protein to the amino-terminus of the protein fusion partner and is designed for use with the Flexi® System, Entry/Transfer (Cat.# C8640) and Flexi® System, Transfer (Cat.# C8820). The vector provides constitutive high-level protein expression in mammalian cells using the human cytomegalovirus (CMV) immediate early enhancer/promoter. The vector 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 Sulfate (Cat.# V7983), under control of an SV40 promoter, allowing selection of stable transfectants.

The pFN28K HaloTag® CMV-neo Flexi® Vector contains the following features:

- **CMV immediate-early enhancer/promoter** for constitutive expression in mammalian cells.
- **T7 RNA polymerase promoter** for in vitro HaloTag® fusion protein expression in cell-free systems (e.g., TnT® lysate reaction).
- **HaloTag® protein coding region**, an engineered tag that rapidly forms covalent bonds with HaloTag® ligands, enabling labeling or immobilization of expressed proteins.
- **HaloTag® linker**, a stretch of amino acids that allows efficient flexibility of the HaloTag® protein when fused to the protein of interest.
- **TEV protease site** for cleavage of the expressed protein from the HaloTag® fusion using HaloTEV Protease (Cat.# G6601).
- The lethal **barnase gene** for positive selection of the insert. **Note:** The pFN28K HaloTag® CMV-neo Flexi® Vector can only be propagated in *E. coli* once the barnase gene is replaced with the protein-coding sequence of interest.
- **Neomycin phosphotransferase gene** for selection of the plasmid in mammalian cells (G-418 resistance) or bacterial cells (kanamycin resistance).
- **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 pFN28K HaloTag® CMV-neo Flexi® Vector from other Flexi® Vectors with different expression options.
- **Synthetic poly(A)** for enhanced translation in eukaryotic systems (in vitro and in vivo).

Concentration: 0.1µg/µl.

GenBank® Accession Number: JN129498.

Storage Buffer: The pFN28K HaloTag® CMV-neo Flexi® Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 8.0).

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.

Note: The insert must contain an in-frame ATG codon for translation initiation.

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.
3. 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 pFN28K HaloTag® CMV-neo Flexi® Vector using the Flexi® System, Entry/Transfer (Cat.# C8640). For more information, see the *Flexi® Vector Systems Technical Manual #*TM254, at: **www.promega.com/protocols/**
4. Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

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: $A_{260}/A_{280} \geq 1.80$.

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 one hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

pFN28K HaloTag® CMV-neo Flexi® Vector Features and Circle Map

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

CMV immediate-early enhancer/promoter	1–742
Chimeric intron	857–989
T7 RNA polymerase promoter (–17 to +3)	1033–1052
HaloTag® N-terminal region	1067–1957
HaloTag® linker	1958–1996
TEV protease site	1970–1990
SgfI site	1997–2004
Barnase coding region	2028–2363
PmeI Site	2365–2372
SV40 late polyadenylation signal	2524–2745
SV40 enhancer and early promoter	2844–3262
EM7 bacterial promoter	3270–3336
Neomycin phosphotransferase coding region	3350–4144
Synthetic polyadenylation signal	4208–4256
Col E 1-derived plasmid origin of replication	4492–4528

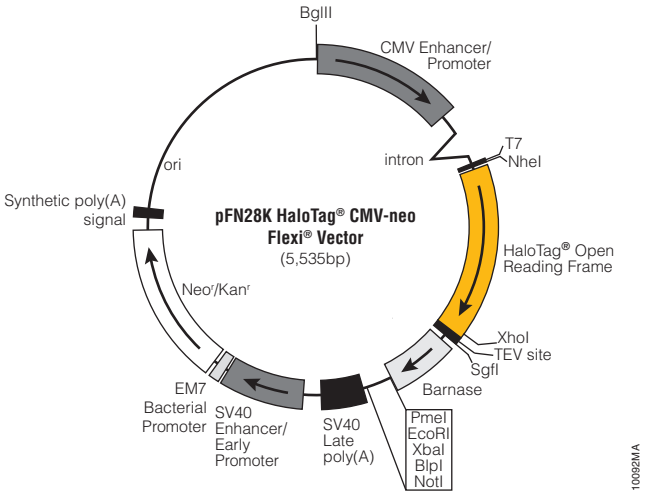

Figure 1. pFN28K HaloTag® CMV-neo Flexi® Vector circle map and sequence reference points.

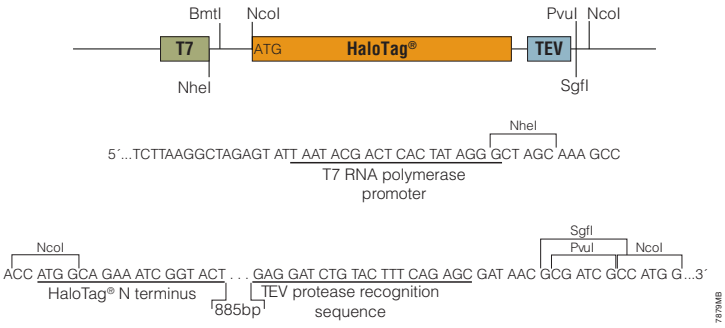

Figure 2. pFN28K HaloTag® CMV-neo Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

Related Products

Product	Size	Cat.#
JM109 Competent Cells, >10^8cfu/µg	5 × 200µl	L2001
JM109 Competent Cells, >10^7cfu/µg	5 × 200µl	L1001
HB101 Competent Cells, >10^8cfu/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
Flexi® System, Entry/Transfer	5 entry and 20 transfer reactions	C8640
Flexi® System, Transfer	100 transfer reactions	C8820

There are Flexi® Vectors available for many different applications.
Visit: **www.promega.com/products/cloning-and-dna-markers/** to learn more.

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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, 8,748,148, 9,416,353, 9,593,316 and other patents and patents pending.

[c]U.S. Pat. Nos. 8,293,503, 9,018,014 and 8,367,403, European Pat. No. 1685247 and other patents and patents pending.

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