Certificate of Analysis

pFC27A HaloTag® CMV-neo Flexi® Vector:

Part No.	Size
G842A	20µg

Part# 9PIG842

Revised 11/21

Description: The pFC27A HaloTag® CMV-neo Flexi Vector[a–d] is configured to append the HaloTag® protein to the carboxy-terminus of the protein fusion partner and is designed for use with the Flexi® System, Entry/Transfer (Cat.# C8640) and Carboxy Flexi® System, Transfer (Cat.# C9320). 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 the control of an SV40 promoter, allowing selection of stable transfectants.

The pFC27A 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 HaloTag® protein when fused to the protein of interest.
- **TEV protease site** for cleavage of the expressed protein from HaloTag® fusion using HaloTEV Protease (Cat.# G6601).
- The lethal **barnase gene** for positive selection of the insert. **Note:** The pFC27A 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.
- **Ampicillin-resistance** gene for selection of the plasmid in *E. coli*.
- **Neomycin phosphotransferase gene** for selection of the plasmid in mammalian cells (G-418 resistance).
- **Unique SgfI and EcoICRI 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 pFC27A HaloTag® CMV-neo Flexi® Vector from other Flexi® Vectors with different expression options.
  Once inserted in this vector, the sequence is no longer available for transfer. For more information, see the *Flexi® Vector Systems Technical Manual* #TM254, available online at: **www.promega.com/protocols/**
- **Synthetic poly(A)** for enhanced translation in eukaryotic systems (in vitro and in vivo).

Concentration: 0.1µg/µl.

GenBank® Accession Number: JN122283.

Storage Buffer: The pFC27A 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.

(continued, next page)

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

Usage Notes: (continued)

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® System, a directional cloning method to shuttle protein-coding sequences between compatible vectors. In this system, carboxy-terminal tag fusions cannot shuttle the insert to other expression vectors. To retain the capacity to transfer a protein-coding sequence to multiple vectors, the insert must first be cloned into any amino-terminal tag kanamycin-resistant Flexi® Vector [e.g., pFN21K CMV Flexi® Vector (Cat.# G2831)] or into an appropriate untagged kanamycin-resistant shuttle vector [e.g., pF4K CMV Flexi® Vector (Cat.# C8491)] using the Flexi® System, Entry/Transfer (Cat.# C8640). Then the protein-coding insert can be transferred to the pFC27A HaloTag® CMV-neo Flexi® Vector using the Carboxy Flexi® System, Transfer (Cat.# C9320). See the *Flexi® Vector Systems Technical Manual* #TM254: **www.promega.com/protocols/**
4. Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

pFC27A 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
SgfI site	1056–1063
Barnase coding region	1087–1422
EcoICRI site	1442–1447
HaloTag® linker	1447–1491
TEV protease site	1462–1482
HaloTag® C-terminal region	1492–2382
SV40 late polyadenylation signal	2516–2737
SV40 enhancer and early promoter	2836–3254
Neomycin phosphotransferase	3299–4093
Synthetic polyadenylation signal	4157–4205
ß-lactamase (Amp^r) coding region	4466–5326
ColE1-derived plasmid origin of replication	5481–5517

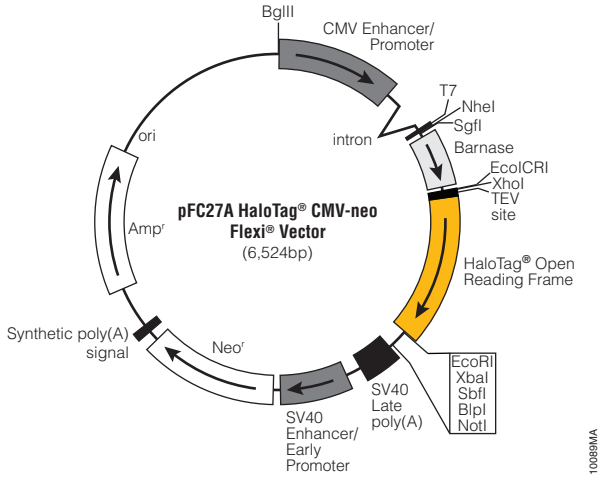

Figure 1. pFC27A HaloTag® CMV-neo Flexi® Vector circle map and sequence reference points.

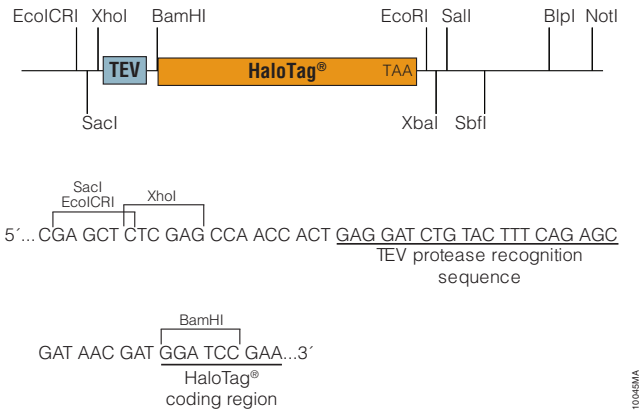

Figure 2. pFC27A HaloTag® CMV-neo Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

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
Carboxy Flexi® System, Transfer	50 transfer reactions	C9320

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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