

Certificate of Analysis

pFC27K HaloTag® CMV-neo Flexi® Vector:

Part No.	Size
G843A	20µg

Part# 9PIG843

Revised 11/21

Description: The pFC27K 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 pFC27K 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 HaloTag® protein using HaloTEV Protease (Cat.# G6601).
- The lethal **barnase gene** for positive selection of the insert. **Note:** The pFC27K 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 EcoCRI 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 pFC27K 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 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: JN122284.

Storage Buffer: The pFC27K 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. After transfection, seed the cells at low density, and add 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.



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Signed by:

R. Wheeler, Quality Assurance

Usage Notes: (continued)

- 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.
- 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., pFN21A CMV Flexi® Vector (Cat.# G2821)] or into an appropriate untagged kanamycin-resistant shuttle vector [e.g., pF4A CMV Flexi® Vector (Cat.# C8481)] using the Flexi® System, Entry/Transfer (Cat.# C8640). Then the protein-coding insert can be transferred to the pFC27K 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/*
- Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

pFC27K 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 region	1462–1482
HaloTag® C-terminal region	1492–2382
SV40 late polyadenylation signal	2516–2737
SV40 enhancer and early promoter	2836–3254
EM7 bacterial promoter	3262–3328
Neomycin phosphotransferase	3342–4136
Synthetic polyadenylation signal	4200–4248
ColE1-derived plasmid origin of replication	4484–4520

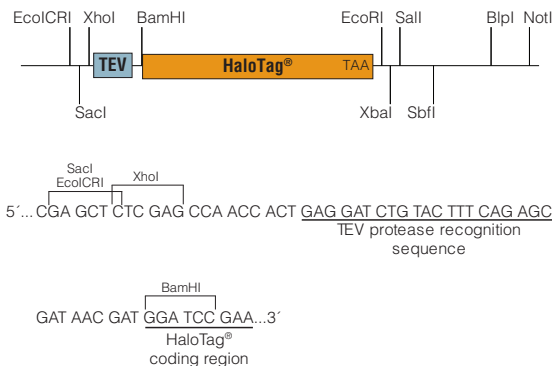


Figure 2. pFC27K HaloTag® CMV-neo Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

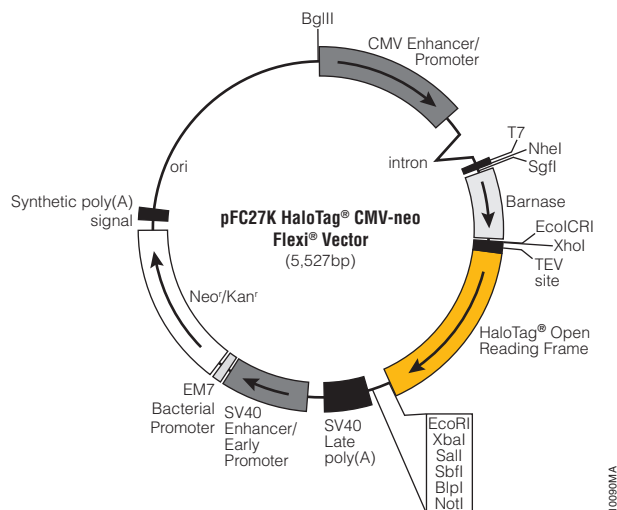


Figure 1. pFC27K HaloTag® CMV-neo Flexi® Vector circle map and sequence reference points.

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
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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