Certificate of Analysis

pFN24K HaloTag® CMV*d3* Flexi® Vector:

Part No.	Size
G298A	20µg

Part# 9PIG298

Revised 4/18

Description: The pFN24K HaloTag® CMV*d3* Flexi® Vector[a–c] is configured to append the HaloTag® tag to the amino-terminus of the protein fusion partner and provides constitutive protein expression in mammalian cells using a modified human cytomegalovirus (CMV) intermediate early enhancer/promoter, CMV*d3*. The vector can be used for both stable and transient gene expression; for stable expression, cotransfection with a vector containing a selectable marker is required.

The pFN24K HaloTag® CMV*d3* Flexi® Vector contains the following features:

- A **modified CMV intermediate-early enhancer/promoter (CMV*d3*)** for constitutive expression in mammalian cells. This modified promoter may provide transient expression lower than the pFN21K HaloTag® CMV, pFN22K HaloTag® CMV*d1* and pFN23K HaloTag® CMV*d2* Flexi® Vectors in many cell types.
- **T7/SP6 RNA polymerase promoters** for in vitro HaloTag® fusion protein expression.
- 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 pFN24K HaloTag® CMV*d3* Flexi® Vector can be propagated only 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 pFN24K HaloTag® CMV*d3* Flexi® Vector from other Flexi® Vectors with different expression options. Once inserted in this vector, the sequence is available for transfer to other Flexi® Vectors. For more information, see the *Flexi® Vector Systems Technical Manual* #TM254, available online at: **www.promega.com/protocols/**

Concentration: 100ng/µl.

GenBank® Accession Number: EU621381.

Storage Buffer: The pFN24K HaloTag® CMV*d3* 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. Use this vector in conjunction with pFN21, pFN22 and pFN23 Flexi® Vectors to determine which vector provides the appropriate protein expression level for your particular application. The pFN21 Flexi® Vector carries the full-length CMV promoter while pFN22, pFN23 and pFN24 Flexi® Vectors contain various deletions of the CMV promoter. Since the full-length CMV promoter expresses highly in many cell types, it may be inappropriate for applications where high concentrations of fusion protein may affect physiological function. See Table 1 on reverse side for additional information.
2. 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 pFN24K HaloTag® CMV*d3* Flexi® Vector using the Flexi® System, Entry/Transfer (Cat.# C8640). For more information, see the *Flexi® Vector Systems Technical Manual* #TM254.
3. 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 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

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Usage Information

pFN24K HaloTag® CMV*d3* Flexi® Vector Features and Circle Map

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

CMV*d3* intermediate early enhancer/promoter	1–66
T7 RNA polymerase promoter (–17 to +3)	77–96
SP6 RNA polymerase promoter (–17 to +3)	101–120
HaloTag® protein coding region	136–1026
TEV site	1039–1059
SgfI site	1066–1073
barnase coding region	1097–1432
PmeI site	1434–1441
SV40 late polyadenylation signal	1593–1814
Kanamycin resistance (Kan^r) coding region	2122–2916
*Col*E1-derived plasmid origin of replication	3085–3121
cer site (site for *E. coli* XerCD recombinase)	3792–4077

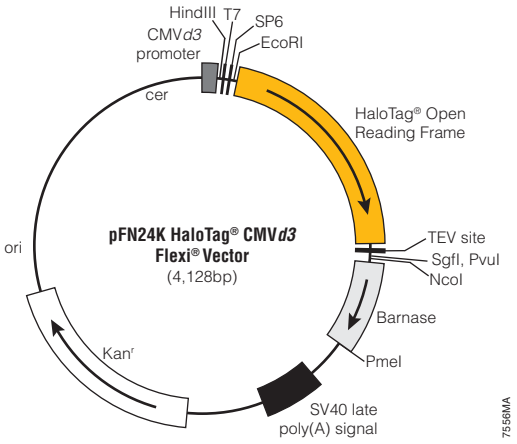

Figure 1. pFN24K HaloTag® CMV*d3* Flexi® Vector circle map and sequence reference points.

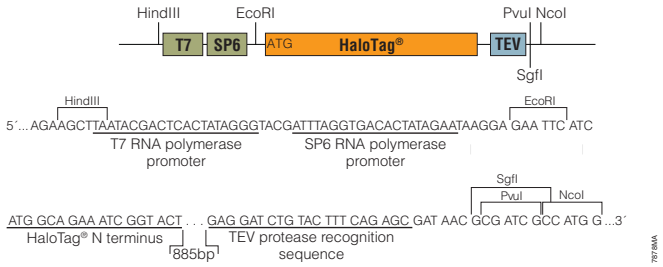

Figure 2. pFN24K HaloTag® CMV*d3* Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

Table 1. Relative Mammalian Expression Levels for HaloTag® Flexi® Vectors.

Vector Name	Cat.#	Expression Level*
pFC14A HaloTag® CMV Flexi® Vector	G9651	High
pFC14K HaloTag® CMV Flexi® Vector	G9661	
pFC15A HaloTag® CMV*d1* Flexi® Vector	G1611	Medium
pFC15K HaloTag® CMV*d1* Flexi® Vector	G1601	
pFC16A HaloTag® CMV*d2* Flexi® Vector	G1591	Low
pFC16K HaloTag® CMV*d2* Flexi® Vector	G1571	
pFC17A HaloTag® CMV*d3* Flexi® Vector	G1551	Ultralow
pFC17K HaloTag® CMV*d3* Flexi® Vector	G1321	
pFN21A HaloTag® CMV Flexi® Vector	G2821	High
pFN21K HaloTag® CMV Flexi® Vector	G2831	
pFN22A HaloTag® CMV*d1* Flexi® Vector	G2841	Medium
pFN22K HaloTag® CMV*d1* Flexi® Vector	G2851	
pFN23A HaloTag® CMV*d2* Flexi® Vector	G2861	Low
pFN23K HaloTag® CMV*d2* Flexi® Vector	G2871	
pFN24A HaloTag® CMV*d3* Flexi® Vector	G2881	Ultralow
pFN24K HaloTag® CMV*d3* Flexi® Vector	G2981	

*Expression level depends on the cell type and the protein fused to HaloTag® coding region.

Related Products

Product	Size	Cat.#
HaloTag® Cloning Starter System		G6050
HaloTag® Flexi® Vectors–CMV Dilution Series	9 × 2µg	G3780
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 (SgfI & PmeI)	25µl	R1851
	100µl	R1852

[a]READ THIS FIRST BEFORE OPENING PRODUCT

The sale of this product and its use are subject to the terms of a limited use label license, the full text of which is available at: **www.promega.com/LULL**. That text must be read by the purchaser prior to opening this product to determine whether the purchaser agrees that all use of the product shall be in accordance with the license terms. If the purchaser is not willing to accept the terms of the limited use label license, Promega is willing to accept the return of the unused product and provide the purchaser with a full refund. However, if the product is opened for any reason, then the purchaser agrees to be bound by the terms of the limited use label license.

[b]U.S. Pat. Nos. 7,425,436 and 7,935,803 and other patents pending.

[c]European Pat. No. 1685247 and other patents pending.

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