Certificate of Analysis

pFC15A HaloTag® CMV*d1* Flexi® Vector:

Part No.	Size
G161A	20µg

Part# 9PIG161

Revised 10/16

Description: The pFC15A HaloTag® CMV*d1* Flexi® Vector[a–d] is configured to append the HaloTag® tag to the carboxy-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*d1*. 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 pFC15A HaloTag® CMV*d1* Flexi® Vector contains the following features:

- A **modified CMV intermediate-early enhancer/promoter (CMV*d1*)** for constitutive expression in mammalian cells. Its expression level is generally lower than that for pFC14A HaloTag® CMV Flexi® Vector but higher than that for the pFC16A HaloTag® CMV*d2* and pFC17A HaloTag® CMV*d3* Flexi® Vectors in many cell types.
- **T7 and SP6 RNA polymerase promoters** for in vitro HaloTag® fusion protein expression in cell-free systems (e.g., TNT® lysate reaction).
- The **C-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 the HaloTag® protein using ProTEV Protease (Cat.# V6051).
- The lethal **barnase gene** for positive selection of the insert. **Note:** The pFC15A HaloTag® CMV*d1* Flexi® Vector can be propagated only in *E. coli* once the barnase gene is replaced with the protein-coding sequence of interest.
- An **ampicillin-resistance gene** for selection of the plasmid.
- 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 pFC15A HaloTag® CMV*d1* 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**

Concentration: 100ng/µl.

GenBank® Accession Number: EU332337.

Storage Buffer: The pFC15A HaloTag® CMV*d1* 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 pFC14, pFC16 and pFC17 Flexi® Vectors to determine which vector provides the appropriate protein expression level for your particular application. The pFC14 Flexi® Vector carries the full-length CMV promoter, while pFC15, pFC16 and pFC17 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.
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. In this system, carboxy-terminal tag fusions cannot be used to shuttle the insert to other expression vectors. To retain the capacity to transfer a protein-coding sequence to multiple vectors, first clone the protein-coding sequence into a kanamycin-resistant Flexi® Vector with no tag or an amino-terminal tag [e.g., pF4K CMV Flexi® Vector (Cat.# C8491) or pFN21K HaloTag® CMV Flexi® Vector (Cat.# G2831)] prior to transferring the insert to the pFC15A HaloTag® CMV*d1* Flexi® Vector. For more information, see the *Flexi® Vector Systems Technical Manual* #TM254, available online at: **www.promega.com/protocols**
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

AF9PIG161 1016G161

Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

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Usage Information

pFC15A HaloTag® CMV*d1* Flexi® Vector Features and Circle Map

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

CMV*d1* intermediate early enhancer/promoter	1–121
T7 RNA polymerase promoter (–17 to +3)	132–151
SP6 RNA polymerase promoter (–17 to +3)	156–175
SgfI site	182–189
barnase coding region	213–548
EcoICRI site	568–573
TEV site	588–608
HaloTag® coding region	618–1508
SV40 late polyadenylation signal	1642–1863
β-lactamase (Amp^r) coding region	2124–2984
*Col*E1-derived plasmid origin of replication	3139–3175
cer site (site for *E. coli* XerCD recombinase)	3846–4131

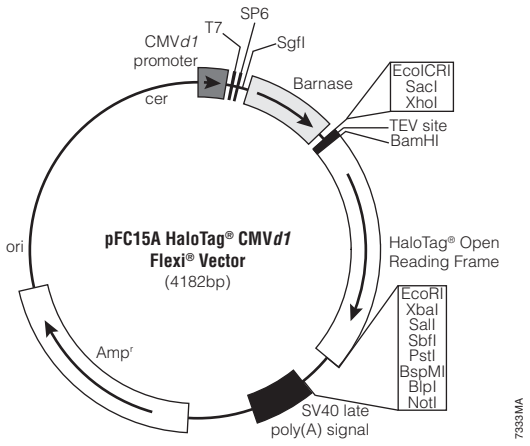

Figure 1. pFC15A HaloTag® CMV*d1* Flexi® Vector circle map and sequence reference points.

Note: Maps of all Flexi® Vectors are available at: **www.promega.com/resources/vector-sequences/**

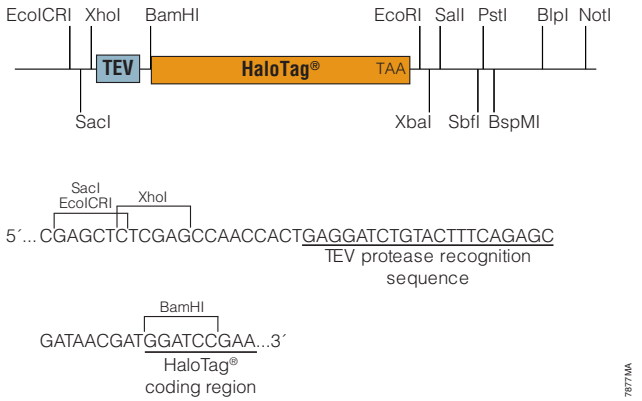

Figure 2. pFC15A HaloTag® CMV*d1* Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

Related Products

Product	Size	Cat.#
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
Carboxy Flexi Enzyme Blend (SgfI & EcoICRI)	50µl	R1901
HaloTag® Flexi® Vectors—CMV Deletion Series Sample Pack	9 × 2µg	G3780
Single Step (KRX) Competent Cells	20 × 50µl	L3002

There are Flexi® Vectors available for many different applications.
Visit: **www.promega.com/applications/cloning/** to find out 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]European Pat. No. 1685247 and other patents pending.

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

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