

## Certificate of Analysis

### pFC16A HaloTag® CMVd2 Flexi® Vector:

<b>Part No.</b>	<b>Size</b>
G159A	20µg

Part# 9PIG159

Revised 7/13

**Description:** The pFC16A HaloTag® CMVd2 Flexi® Vector<sup>(a-d)</sup> 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, CMVd2. 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 pFC16A HaloTag® CMVd2 Flexi® Vector contains the following features:

- A **modified CMV intermediate-early enhancer/promoter (CMVd2)** for constitutive expression in mammalian cells. Its expression level is generally lower than pFC14A HaloTag® CMV and pFC15A HaloTag® CMVd1 Flexi® Vectors but higher than the pFC17A HaloTag® CMVd3 Flexi® Vector in many cell types.
- **T7/SP6 RNA polymerase promoters** for in vitro HaloTag® fusion protein expression in cell-free systems (e.g., TnT<sup>®</sup> 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 HaloTag® using ProTEV Protease (Cat.# V6051).
- The lethal **barnase gene** for positive selection of the insert. **Note: The pFC16A HaloTag® CMVd2 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 EcoRI 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 pFC16A HaloTag® CMVd2 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](http://www.promega.com/protocols)

**Concentration:** 100ng/µl.

**GenBank® Accession Number:** EU332339.

**Storage Buffer:** The pFC16A HaloTag® CMVd2 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, pFC15 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 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 pFC16A HaloTag® CMVd2 Flexi® Vector. For more information, see the *Flexi® Vector Systems Technical Manual #TM254*, available online at: [www.promega.com/protocols](http://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/](http://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.



## 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	<a href="http://www.promega.com">www.promega.com</a>

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Signed by:

J. Stevens, Quality Assurance

## pFC16A HaloTag® CMVd2 Flexi® Vector Features and Circle Map

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

CMVd2 intermediate early enhancer/promoter	1–73
T7 RNA polymerase promoter (–17 to +3)	84–103
SP6 RNA polymerase promoter (–17 to +3)	108–127
Sgfl site	134–141
barnase coding region	165–500
EcoICRI site	520–525
TEV site	540–560
HaloTag® coding region	570–1460
SV40 late polyadenylation signal	1594–1815
β-lactamase (Amp <sup>r</sup> ) coding region	2076–2936
ColE1-derived plasmid origin of replication	3091–3127
cer site (site for <i>E. coli</i> XerCD recombinase)	3798–4083

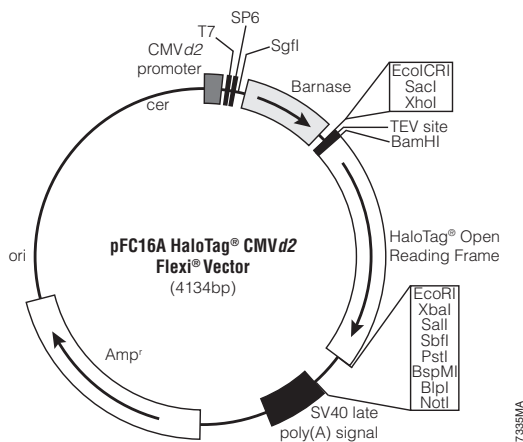


Figure 1. pFC16A HaloTag® CMVd2 Flexi® Vector circle map and sequence reference points.

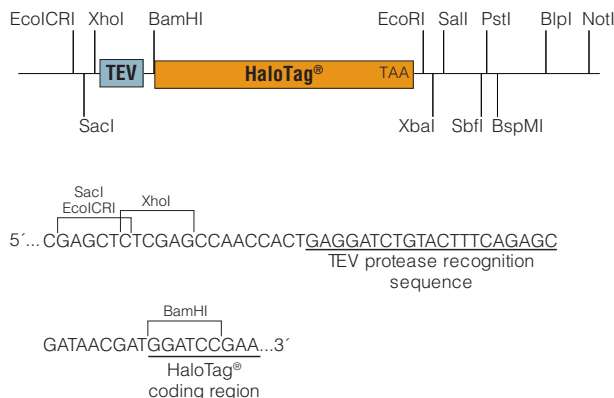


Figure 2. pFC16A HaloTag® CMVd2 Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

## 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 (Sgfl & Pmel)	25µl	R1851
	100µl	R1852
Carboxy Flexi Enzyme Blend (Sgfl & EcoICRI)	50µl	R1901
HaloTag® Flexi® Vectors—CMV Dilution Series Sample Pack	9 × 2µg	G3780
Single Step (KRX) Competent Cells	5 × 200µl	L3001

There are Flexi® Vectors available for many different applications. Visit: [www.promega.com/applications/cloning](http://www.promega.com/applications/cloning) to find out more.

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