Certificate of Analysis

pFC14A HaloTag® CMV Flexi® Vector:

Part No.	Size
G965A	20µg

Part# 9PIG965

Revised 10/16

Description: The pFC14A HaloTag® CMV Flexi® Vector[a–c] 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 the human cytomegalovirus (CMV) intermediate early enhancer/promoter. 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 pFC14A HaloTag® CMV Flexi® Vector contains the following features:

- A **CMV intermediate/early enhancer/promoter** for constitutive expression in mammalian cells.
- A **T7 RNA polymerase promoter** 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 HaloTag® using ProTEV Protease (Cat.# V6051).
- The lethal **barnase gene** for positive selection of the insert. **Note: The pFC14A HaloTag® CMV 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 pFC14A HaloTag® CMV 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: EU113046.

Storage Buffer: The pFC14A HaloTag® CMV 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 pFC15, 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 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 pFC14A HaloTag® CMV 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

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Usage Information

pFC14A HaloTag® CMV Flexi® Vector Features and Circle Map

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

CMV intermediate 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
TEV site	1462–1482
HaloTag® coding region	1492–2382
SV40 late polyadenylation signal	2516–2737
β-lactamase (Amp^r) coding region	2998–3858
*Col*E1-derived plasmid origin of replication	4013–4049
cer site (site for *E. coli* XerCD recombinase)	4720–5005

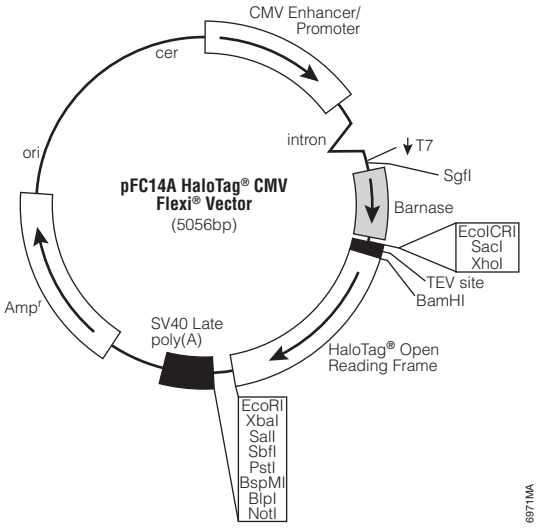

Figure 1. pFC14A HaloTag® CMV Flexi® Vector circle map and sequence reference points.

Note: Maps of all the Flexi® Vectors are available at: **www.promega.com/vectors**

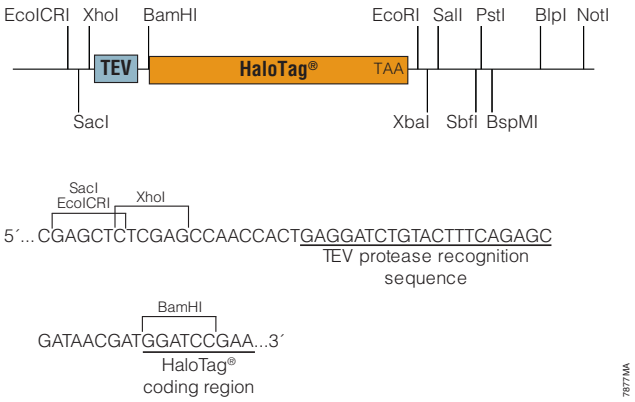

Figure 2. pFC14A HaloTag® CMV Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

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 Dilution Series	9 × 2µg	G3780
Single Step (KRX) Competent Cells	5 x 200µl	L3001

There are Flexi® Vectors available for many different applications.
Visit: **www.promega.com/applications/cloning** to find out more.

[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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