Certificate of Analysis

pFC20K HaloTag® T7 SP6 Flexi® Vector:

Part No.	Size
G169A	20µg

Part# 9PIG169

Revised 10/16

Description: The pFC20K HaloTag® T7 SP6 Flexi® Vector[a–d] is configured to append the HaloTag® tag to the carboxy-terminus of the protein fusion partner and provides T7 RNA polymerase-driven protein expression in *E. coli* or T7 or SP6 RNA polymerase-driven protein expression in cell-free translation systems.

The pFC20K HaloTag® T7 SP6 Flexi® Vector contains the following features:

- **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 pFC20K HaloTag® T7 SP6 Flexi® Vector can only be propagated 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 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 pFC20K HaloTag® T7 SP6 Flexi® Vector from other Flexi® Vectors with different expression options. **Once inserted in this vector, the sequence is no longer available for transfer**.
- A **synthetic poly(A)** for enhanced translation in eukaryotic cell-free translation systems.
- A ***rrnB* transcription terminator** for preventing in vivo *E. coli* transcription into the insert.

Concentration: 100ng/µl.

GenBank® Accession Number: EU545997.

Storage Buffer: The pFC20K HaloTag® T7 SP6 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. 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 an ampicillin-resistant Flexi® Vector with no tag or an amino-terminal tag [e.g., pF4A CMV Flexi® Vector (Cat.# C8481) or pFN21A HaloTag® CMV Flexi® Vector (Cat.# G2821)] prior to transferring the insert to the pFC20K HaloTag® T7 SP6 Flexi® Vector. For more information, see the *Flexi® Vector Systems Technical Manual* #TM254, available online at: **www.promega.com/protocols**
2. 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

AF9PIG169 1016G169

Promega

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Usage Information

pFC20K HaloTag® T7 SP6 Flexi® Vector Features and Circle Map

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

T7 RNA polymerase promoter (-17 to +3)	21–40
SP6 RNA polymerase promoter (-17 to +3)	45–64
SgfI site	71–78
barnase coding region	102–437
EcoICRI site	457–462
TEV site	477–497
HaloTag® protein coding region	507–1397
Synthetic poly(A) region	1510–1539
T7 terminator	1540–1587
Kanamycin resistance (Kan^r) coding region	1968–2762
*Col*E1-derived plasmid origin of replication	2931–2967
cer site (site for *E. coli* XerCD recombinase)	3638–3923
rrnB transcription terminator	3974–4375

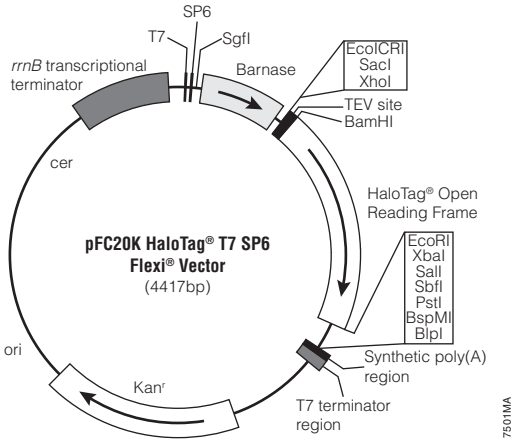

Figure 1. pFC20K HaloTag® T7 SP6 Flexi® Vector circle map and sequence reference points.

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

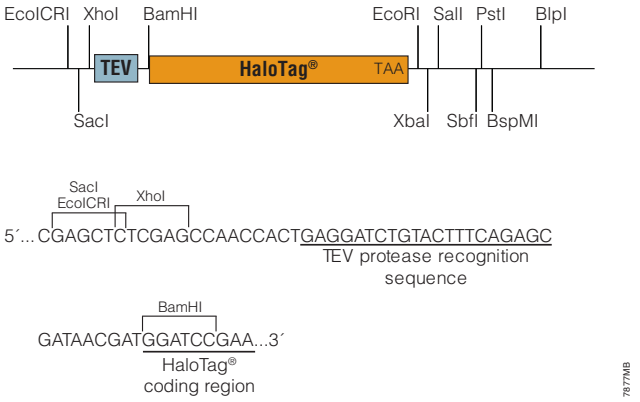

Figure 2. pFC20K HaloTag® T7 SP6 Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

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
Wizard® SV Gel and PCR Clean-Up System	50 preps	A9281
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.

Summary of Changes

The following changes were made to the 12/14 revision of this document:

1. Expired patent or license statements were removed.

[a]BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE STATEMENT. If the researcher is not willing to accept the conditions of this limited use statement, and the product is unused, Promega will accept return of the unused product and provide the researcher with a full refund.

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]U.S. Pat. Nos. 7,425,436, 7,935,803, 8,466,269, 8,742,086, 8,420,367 and 8,748,148 and other patents and patents pending.

[c]U.S. Pat. Nos. 8,293,503 and 8,367,403, European Pat. No. 1685247 and other patents and patents pending.

[d]For research use only. Persons wishing to use this product or its derivatives in other fields of use, including without limitation, commercial sale, diagnostics or therapeutics, should contact Promega Corporation for licensing information.

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