Certificate of Analysis

pFC20A HaloTag® T7 SP6 Flexi® Vector:

Part No.	Size
G168A	20µg

Part# 9PIG168

Revised 4/18

Description: The pFC20A 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 pFC20A 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 pFC20A 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.
- 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 pFC20A 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: EU545996.

Storage Buffer: The pFC20A 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 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 the pFC20A 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

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Usage Information

pFC20A 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
β-lactamase (Amp^r) coding region	1921–2781
*Col*E1-derived plasmid origin of replication	2936–2972
cer site (site for *E. coli* XerCD recombinase)	3643–3928
rrnB transcription terminator	3979–4380

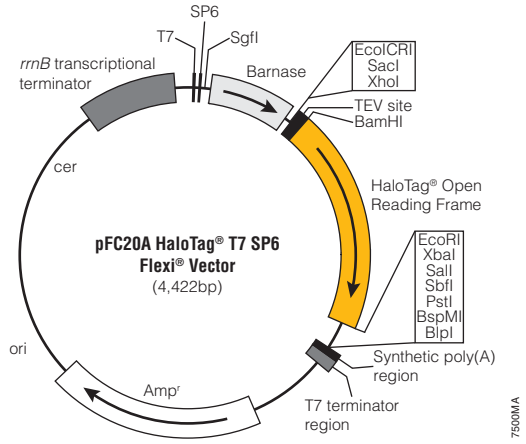

Figure 1. pFC20A HaloTag® T7 SP6 Flexi® Vector circle map and sequence reference points.

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

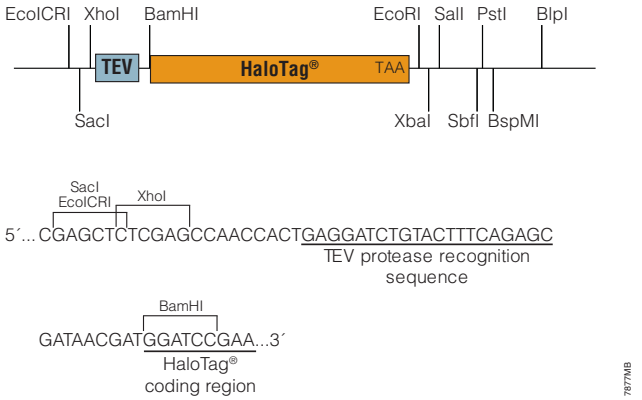

Figure 2. pFC20A HaloTag® T7 SP6 Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

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

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