Certificate of Analysis

pFC30A His_6HaloTag® T7 Flexi® Vector:

Part No.	Size
G832A	20µg

Part# 9PIG832

Revised 10/16

Description: The pFC30A His_6HaloTag® T7 Flexi® Vector[a–d] is configured to append the His_6HaloTag® tag to the carboxy-terminus of the protein fusion partner and provides T7 RNA polymerase-driven protein expression in *E. coli*.

The pFC30A His_6HaloTag® T7 Flexi® Vector contains the following features:

- **T7 RNA polymerase promoters** for in vitro HaloTag® fusion protein expression in cell-free systems (e.g., TnT® Lysate reaction) and in vivo expression in *E. coli* strains containing T7 RNA polymerase.
- The **C-terminal His_6HaloTag® region**, which allows simple purification via the hexahistidine tag and rapid formation of covalent bonds with HaloTag® ligands and surfaces, enabling labeling and immobilization of expressed proteins.
- A **TEV protease site** for cleavage of the expressed protein from His_6HaloTag® using HaloTEV Protease (Cat.# G6601).
- The lethal **barnase gene** for positive selection of the insert. **Note: The pFC30A His_6HaloTag® T7 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 pFC30A His_6HaloTag® T7 Flexi® Vector from other Flexi® Vectors with different expression options. **Once inserted in this vector, the sequence is no longer available for transfer**.
- A ***rrn*B transcription terminator** for preventing in vivo *E. coli* transcription into the insert.

Concentration: 100ng/µl.

GenBank® Accession Number: JN874649.

Storage Buffer: The pFC30A His_6HaloTag® T7 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 Note: 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 pFC30A His_6HaloTag® T7 Flexi® Vector. For more information, see the *Flexi® Vector Systems Technical Manual* #TM254, available online at: **www.promega.com/resources/protocols/**

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/products/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

AF9PIG832 1016G832

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Part# 9PIG832
Printed in USA. Revised 10/16.

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Usage Information

pFC30A His$_6$HaloTag® T7 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
SgfI region	61–68
EcoICRI region	447–452
HaloTag® linker region	452–496
TEV protease region	467–487
HaloTag® region	497–1387
His$_6$HaloTag® protein coding region	497–1405
His$_6$ region	1388–1405
T7 terminator region	1430–1477
β-lactamase (Amp^r) coding region	1811–2671
*Col*E1-derived plasmid origin of replication	2826–2862
*rrn*B transcription terminator	3869–4270

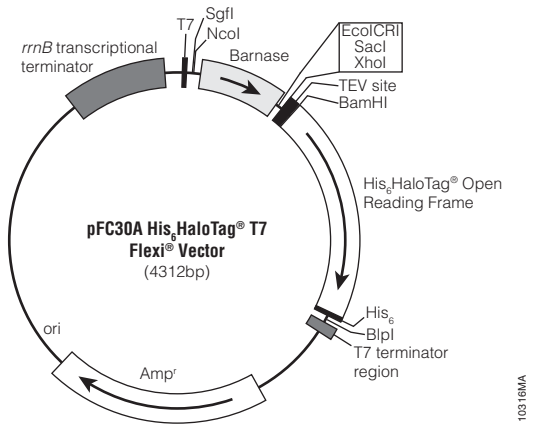

Figure 1. pFC30A His$_6$HaloTag® T7 Flexi® Vector circle map and sequence reference points.

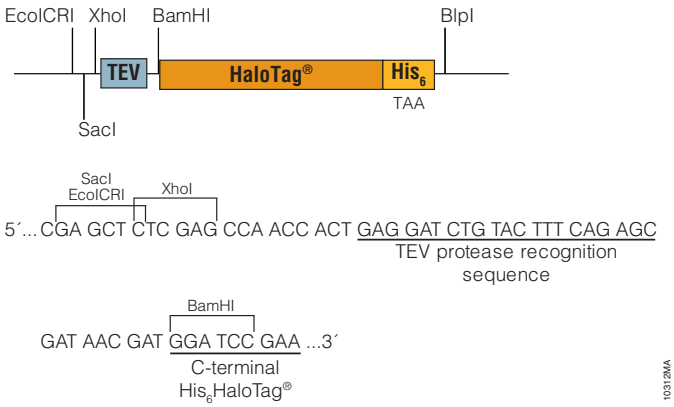

Figure 2. pFC30A His$_6$HaloTag® T7 Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

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
Single Step (KRX) Competent Cells	20 x 50µl	L3002
ProTEV Plus	1,000 units	V6101
HaloTEV Protease	1,000 units	G6601
	4,000 units	G6602

There are Flexi® Vectors available for many applications.
Visit: **www.promega.com/products/protein-expression-and-analysis/** 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.

Part# 9PIG832
Printed in USA. Revised 10/16.