Certificate of Analysis

pFN29A His_6HaloTag® T7 Flexi® Vector:

Part No.	Size
G826A	20µg

Part# 9PIG826

Revised 10/16

Description: The pFN29A His_6HaloTag® T7 Flexi® Vector[a–d] is configured to append the His_6HaloTag® tag to the amino-terminus of the protein fusion partner and provides T7 RNA polymerase-driven protein expression in *E. coli*. The vector contains a His_6HaloTag® protein coding region that allows for both purification and labeling of the expressed fusion protein.

The pFN29A His_6HaloTag® T7 Flexi® Vector contains the following features:

- A **T7 RNA polymerase promoter** for in vitro HaloTag® fusion protein expression in cell-free systems (e.g., E. coli T7 S30 Extract System for Circular DNA) and in vivo expression in *E. coli* strains containing T7 RNA polymerase.
- The **N-terminal His_6HaloTag® region**, which allows simple purification via the hexahistidine tag and also allows rapid formation of covalent bonds with HaloTag® ligands and surfaces, allowing 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 pFN29A 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 PmeI 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 pFN29A His_6HaloTag® T7 Flexi® Vector from other Flexi® Vectors with different expression options.
- A ***rrn*B transcription terminator** for preventing in vivo *E. coli* transcription into the insert.

Concentration: 100ng/µl.

GenBank® Accession Number: JN874648.

Storage Buffer: The pFN29A 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. 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. To prepare the HaloTag® fusion protein, the protein coding region is cloned into the pFN29A His_6HaloTag® T7 Flexi® Vector using the Flexi® System, Entry/Transfer (Cat.# C8640). 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/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

AF9PIG826 1016G826

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Usage Information

pFN29A 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
His$_6$HaloTag® protein coding region	70–981
His$_6$ region	76–93
HaloTag® region	94–981
HaloTag® linker region	982–1020
TEV protease region	994–1014
SgfI region	1021–1028
PmeI region	1389–1396
T7 terminator region	1516–1563
β-lactamase (Amp^r) coding region	1897–2757
*Col*E1-derived plasmid origin of replication	2912–2948
*rrn*B transcription terminator	3955–4356

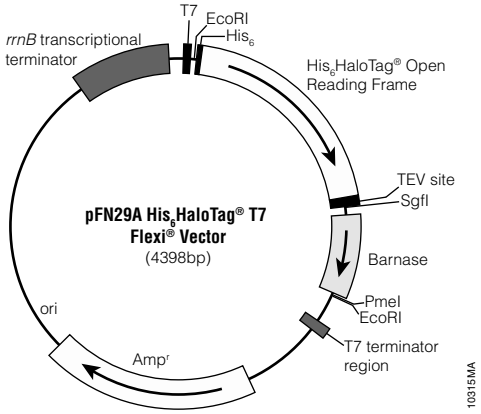

Figure 1. pFN29A His$_6$HaloTag® T7 Flexi® Vector circle map and sequence reference points.

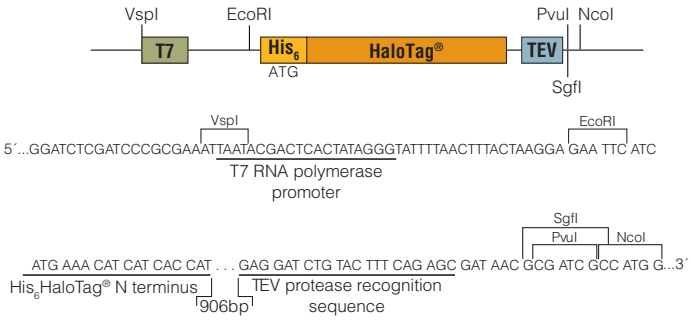

Figure 2. pFN29A His$_6$HaloTag® T7 Flexi® Vector sequence upstream and downstream of the HaloTag® gene.

Related Products

Product	Size	Cat.#
HaloTag® Cloning Starter System	1 each	G6050
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 × 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.

[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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