

Certificate of Analysis

pFN29A His₆HaloTag[®] T7 Flexi[®] Vector:

| | |
|-----------------|-------------|
| Part No. | Size |
| G826A | 20µg |

Description: The pFN29A His₆HaloTag[®] T7 Flexi[®] Vector^(a-d) is configured to append the His₆HaloTag[®] 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 His₆HaloTag[®] protein coding region that allows for both purification and labeling of the expressed fusion protein.

The pFN29A His₆HaloTag[®] 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 His₆HaloTag[®] 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 His₆HaloTag[®] using HaloTEV Protease (Cat.# G6601).
- The lethal **barnase gene** for positive selection of the insert. **Note: The pFN29A His₆HaloTag[®] 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 His₆HaloTag[®] T7 Flexi[®] Vector from other Flexi[®] Vectors with different expression options.
- A **rrb transcription terminator** for preventing in vivo *E. coli* transcription into the insert.

Concentration: 100ng/µl.

GenBank[®] Accession Number: JN874648.

Storage Buffer: The pFN29A His₆HaloTag[®] 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 His₆HaloTag[®] 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.

Part# 9PIG826

Revised 10/16



AF9PIG826 1016G826



Promega Corporation

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All specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products

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Signed by:

R. Wheeler, Quality Assurance

pFN29A His₆HaloTag[®] 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 |
| Sgfl 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 |
| <i>rrnB</i> transcription terminator | 3955-4356 |

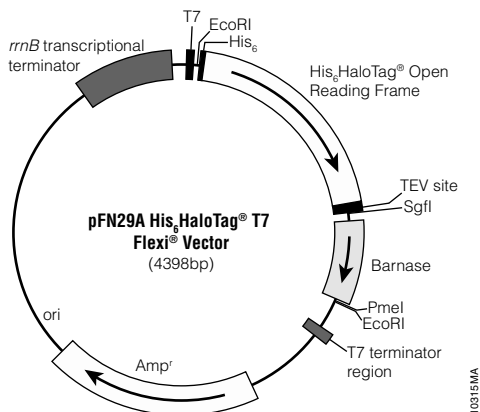


Figure 1. pFN29A His₆HaloTag[®] T7 Flexi[®] Vector circle map and sequence reference points.

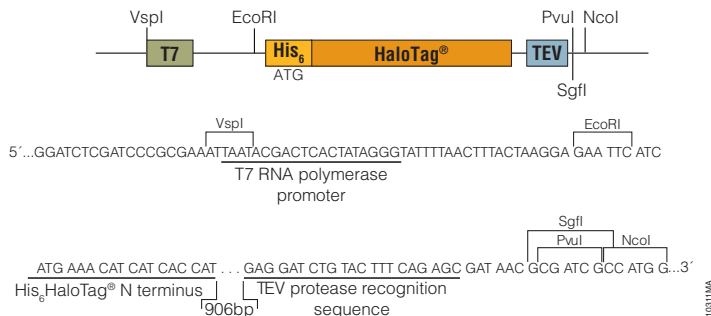


Figure 2. pFN29A His₆HaloTag[®] T7 Flexi[®] Vector sequence upstream and downstream of the HaloTag[®] gene.

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 & EcoRI) | 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.

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