

Certificate of Analysis

pFN2A (GST) Flexi® Vector:

Part No.	Size (units)
C846A	20µg

Description: The pFN2A (GST) Flexi® Vector^(a-c) is designed for use with the Flexi® System, Entry/Transfer (Cat.# C8640), and the Flexi® System, Transfer (Cat.# C8820). The vector contains a T7 promoter for bacterial or in vitro protein expression of a protein-coding region. The vector appends an N-terminal glutathione-S-transferase (GST) coding region that can be used to purify the expressed protein. The GST tag contains a TEV protease site for removal of the tag after purification. The vector also contains the lethal barnase gene for positive selection of the insert, an ampicillin-resistance gene for selection of the plasmid and unique SgfI and PmeI sites that allow easy insertion or transfer of the sequence of interest. Inserts containing a protein-coding region can easily be transferred from the pFN2A (GST) Flexi® Vector to other Flexi® Vectors with different expression options (Table 1). For more information, see the *Flexi® Vector Systems Technical Manual #TM254*.

Table 1. Vectors Available for Use With the Flexi® Vector Systems.

Cat.#	Flexi® Vector	Utility	Expression	Drug Selection
C8441	pF1A T7 Flexi® Vector	Protein expression	<i>E. coli</i> and in vitro (T7 promoter)	Ampicillin
C8451	pF1K T7 Flexi® Vector			Kanamycin
C8461	pFN2A (GST) Flexi® Vector	Protein expression	<i>E. coli</i> and in vitro (T7 promoter)	Ampicillin
C8471	pFN2K (GST) Flexi® Vector	and purification		Kanamycin
L5671	pF3A WG (BYDV) Flexi® Vector	Protein expression	Wheat Germ in vitro (T7, SP6)	Ampicillin
L5681	pF3K WG (BYDV) Flexi® Vector	Protein expression	Wheat Germ in vitro (T7, SP6)	Kanamycin
C8481	pF4A CMV Flexi® Vector	Protein expression	Mammalian (CMV promoter)	Ampicillin
C8491	pF4K CMV Flexi® Vector		and in vitro (T7 promoter)	Kanamycin

Usage Information

Concentration: 100ng/µl.

GenBank® Accession Number: AY753578.

Storage Buffer: The pFN2A (GST) Flexi® Vector is supplied in 10mM Tris-HCl (pH 8.0), 1mM EDTA.

Storage Conditions: Store the vector at -20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

Usage Notes: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

Quality Control Assays

Nuclease Assay: Following incubation of 1µg of pFN2A (GST) Flexi® Vector in Restriction Enzyme Buffer B at 37°C for 16 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

Physical Purity: $A_{260}/A_{280} > 1.80$.

Restriction Digestion: The presence of unique restriction sites for PmeI and SgfI is confirmed by showing that the vector yields the expected fragment sizes after digesting 1µg of vector for 2 hours with 10 units of PmeI, SgfI and BgIII.

^(a)Patent Pending.

^(b)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.

^(c)U.S. Pat. Nos. 8,293,503 and 8,367,403, European Pat. No. 1685247 and other patents and patents pending.

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIC846

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pFN2A (GST) 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
GST coding region	70-723
TEV protease site	742-762
Sgfl site	760-767
barnase coding region	791-1126
PmeI site	1128-1135
T7 terminator	1255-1302
β-lactamase (Amp ^r) coding region	1636-2496
ColE1-derived plasmid origin of replication	2651-2687
cer site (site for <i>E. coli</i> XerCD recombinase)	3358-3643
rrnB transcription terminator	3694-4095

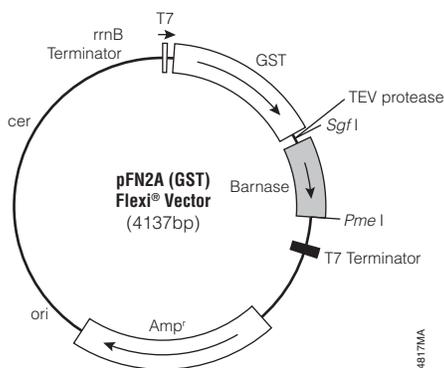


Figure 1. pFN2A (GST) Flexi® Vector circle map and sequence reference points.

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 (Sgfl & PmeI)	25µl	R1851
	100µl	R1852
Carboxy Flexi® Enzyme Blend (Sgfl & EcoICRI)	50µl	R1901
HaloTag® Flexi® Vectors-CMV Dilution Series Sample Pack	9 × 2µg	G3780
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 8/14 and 12/14 revisions of this document:

1. Expired patent or license statements were removed.