

Certificate of Analysis

pFN2A (GST) Flexi® Vector:

Part No.	Size (units)
C846A	20µg

Description: The pFN2A (GST) Flexi® Vector^(a,b,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 Bgl II.

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Signed by:

J. Stevens, Quality Assurance

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
SgfI 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

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 & EcolCRI)	50µl	R1901
HaloTag® Flexi® Vectors—CMV Dilution Series Sample Pack	9 × 2µg	G3780
Single Step (KRX) Competent Cells	5 x 200µl	L3001

There are Flexi® Vectors available for many different applications.
Visit: www.promega.com/applications/cloning to find out more.

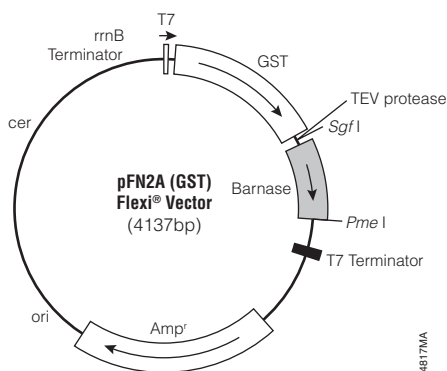


Figure 1. pFN2A (GST) Flexi® Vector circle map and sequence reference points.

^(a)Patent Pending.

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