Product Contents

pF5K CMV-neo Flexi® Vector:

Part No.	Size (units)
C941A	20µg

Description: The pF5K CMV-neo 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 pF5K CMV-neo Flexi® Vector contains a CMV immediate early enhancer/promoter region plus a chimeric intron for mammalian expression and a T7 promoter for in vitro expression of the protein-coding region. The vector also contains the barnase gene for positive selection of the insert, and unique SgfI and PmeI sites that allow easy insertion and transfer of the insert of interest. The pF5K CMV-neo Flexi® Vector also contains a neomycin resistance gene with dual bacterial and SV40 promoters for kanamycin resistance and selection of the plasmid in *E. coli* and for long-term selection capabilities in mammalian cells. Transfection of a pF5K CMV-neo Flexi® Vector containing a cloned protein-coding region into mammalian cells provides resistance to the antibiotic G-418 (Cat.# V7981, V8091), allowing long-term selection of transfectants. **Do not use the pF5K CMV-neo Flexi® Vector without an insert as a negative control** because the barnase gene decreases the viability of the transfected cells. Cloned protein-coding regions can be transferred from the pF5K CMV-neo Flexi® Vector to other Flexi® Vectors with different expression options using the Flexi® Entry/Transfer Systems (Cat.# C8640 and C8820). For more information, see the *Flexi® Vector Systems Technical Manual* #TM254.

Usage Information

Concentration: 100ng/µl.

GenBank® Accession Number: DQ487156.

Storage Buffer: The pF5K CMV-neo 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.

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 pF5K CMV-neo 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: $A_{260}/A_{280} \geq 1.80$.

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

[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]The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

Part# 9PIC941

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Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

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Usage Information

pF5K CMV-neo Flexi® Vector Features and Circle Map

The following features are present in the vector based on nucleotide sequence.

CMV immediate early enhancer/promoter	1–742
chimeric intron	857–989
T7 RNA polymerase promoter (–17 to +3)	1033–1052
SgfI site	1056–1063
barnase coding region	1087–1422
PmeI site	1424–1431
SV40 late poly(A) signal	1583–1804
SV40 enhancer and early promoter	1903–2320
EM7 bacterial promoter	2337–2394
neomycin phosphotransferase coding region	2409–3203
synthetic polyadenylation signal	3267–3313
ColE1-derived plasmid replication of origin	3551–3587
cer site (site for *E. coli* XerCD recombinase)	4258–4543

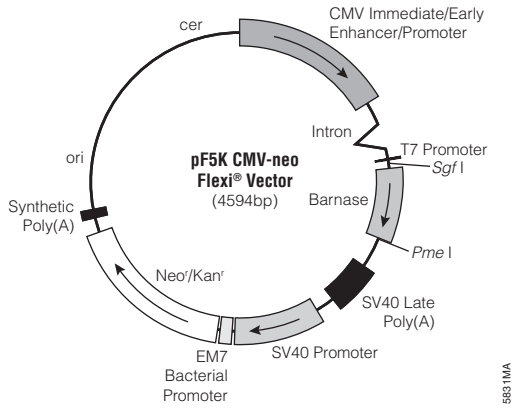

Figure 1. pF5K CMV-neo Flexi® Vector circle map.

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
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.

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