

Certificate of Analysis

pF4K CMV Flexi® Vector:

Part No. Size (units)
C849A 20µg

Description: The pF4K CMV Flexi® Vector^(a,b,d) is designed for use with the Flexi® System, Entry/Transfer (Cat.# C8640), and the Flexi® System, Transfer (Cat.# C8820). The vector contains a CMV immediate early enhancer/promoter plus a chimeric intron for mammalian expression of the protein-coding region and a T7 promoter for in vitro protein expression of the protein-coding region. The vector also contains the lethal barnase gene for positive selection of the insert, a kanamycin-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 pF4K CMV 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: AY753581.

Storage Buffer: The pF4K CMV 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 pF4K CMV 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 vector for 2 hours with 10 units of PmeI, SgfI and Bgl II.

^(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.

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIC849

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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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pF4K CMV 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
Sgfl site	1056–1063
barnase coding region	1087–1422
PmeI site	1424–1431
SV40 late poly(A) signal	1583–1804
kanamycin resistance coding region	2112–2906
ColE1-derived plasmid origin of replication	3075–3111
cer site (site for <i>E. coli</i> XerCD recombinase)	3782–4067

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

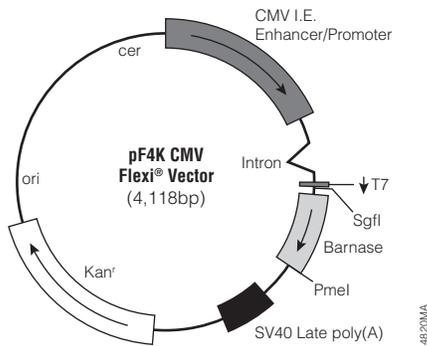


Figure 1. pF4K CMV Flexi® Vector circle map and sequence reference points.