

Product Contents

pFN10A (ACT) Flexi® Vector:

Part No. Size (units)
C933A 20µg



Instructions for use of this product can be found in the CheckMate™/Flexi® Vector Mammalian Two-Hybrid System Technical Manual #TM283, available online at: www.promega.com/tbs

Description: The pFN10A (ACT) Flexi® Vector^(a,b,c) is designed for use with the CheckMate™/Flexi® Vector Mammalian Two-Hybrid System (Cat.# C9360), the Flexi® System, Entry/Transfer (Cat.# C8640) and the Flexi® System, Transfer (Cat.# C8820). The pFN10A (ACT) Flexi® Vector contains a herpes simplex virus VP16 activation domain upstream and in-frame of the cloning site. The pFN10A (ACT) Flexi® Vector also contains a neomycin resistance gene under the control of the SV40 promoter for long-term selection of transfected mammalian cells. Transfection of a pFN10A (ACT) Flexi® Vector containing a cloned protein-coding region into mammalian cells provides resistance to the antibiotic G-418 (Cat.# V7981 or V8091) and allows selection of transfectants. In common with other Flexi® Vectors, the pFN10A (ACT) Flexi® Vector contains a CMV immediate early enhancer/promoter region plus a chimeric intron for mammalian expression, a T7 promoter for in vitro expression of the protein-coding region, a 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 and transfer of the insert of interest. **Do not use the pFN10A (ACT) Flexi® Vector without an insert as a negative control** because the presence of the barnase gene decreases the viability of the transfected cells. The pFN10A (ACT) Flexi® Vector containing a cloned protein-coding region can be used to transfer the insert to other Flexi® Vectors with different expression options using the Flexi® Systems. For more information, see the *CheckMate™/Flexi® Vector Mammalian Two-Hybrid System Technical Manual #TM283* and the *Flexi® Vector Systems Technical Manual #TM254*.

Concentration: 100ng/µl.

GenBank® Accession Number: DQ487211.

Storage Buffer: The pFN10A (ACT) Flexi® Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 8.0 at 25°C).

Storage Conditions: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Usage Notes: Mix well prior to use.

Quality Control Assays

Nuclease Assay: Following incubation of 1µg of pFN10A (ACT) 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)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. Research Use includes contract research for which monetary or other consideration may be received. Other commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

^(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)Patent Pending.

Part# 9PIC933

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pFN10A (ACT) Flexi® Vector Features and Circle Map

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

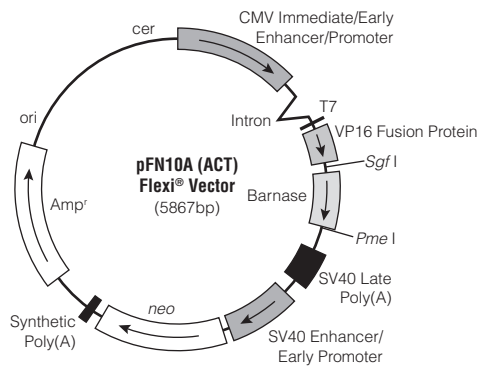
CMV immediate/early enhancer/promoter	1–742
Chimeric intron	857–989
T7 RNA polymerase promoter (–17 to +3)	1033–1052
VP16 fusion protein	1083–1292
IgA linker	1299–1340
SgfI site	1332–1339
Barnase coding region	1363–1698
PmeI site	1700–1706
SV40 late polyadenylation signal	1859–2080
SV40 enhancer/early promoter	2179–2597
Neomycin phosphotransferase coding region	2642–3436
Synthetic polyadenylation signal	3500–3548
β-lactamase (Amp ^r) coding region	3809–4669
ColE1-derived plasmid origin of replication	4824–4860
cer region (site for <i>E. coli</i> XerCD recombinase)	5531–5816

Note: The IgA linker encodes the polypeptide AIPSTPPTSPAIA.

Related Products

Product	Size	Cat. #
Flexi® System, Entry/Transfer	5 entry/20 transfer reactions	C8640
Single Step (KRX) Competent Cells	5 × 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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Figure 1. pFN10A (ACT) Flexi® Vector circle map and sequence reference points.

Maps of all the Flexi® Vectors are available at:
www.promega.com/vectors/cloning_vectors.htm