

Certificate of Analysis

pF9A CMV *hRluc*-neo Flexi[®] Vector:

Part No. Size
C936A 20µg

Description: The pF9A CMV *hRluc*-neo Flexi[®] Vector^(a-e) 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 region plus a chimeric intron for mammalian expression, and a T7 promoter for in vitro expression of the protein-coding region. In addition, the vector contains a barnase gene for positive selection of the insert, an ampicillin resistance gene for selection of the plasmid and unique SgfI and Pml sites that allow easy insertion and transfer of the insert of interest. The pF9A CMV *hRluc*-neo Flexi[®] Vector also contains a unique neomycin resistance gene fused with a humanized *Renilla* luciferase (*hRluc*) gene, under the control of the SV40 promoter, allowing long-term selection of transfected mammalian cells and the ability to monitor those selected cells using a *Renilla* luciferase assay. Transfection of the pF9A CMV *hRluc*-neo Flexi[®] Vector containing a protein-coding region into mammalian cells provides resistance to the antibiotic G-418 (Cat.# V7981 or V8091) and allows selection of transfectants. With some cell types it may be beneficial to use a lower concentration of G-418 for initial selection of resistant clones. **Do not use the pF9A CMV *hRluc*-neo Flexi[®] Vector without an insert as a negative control** because the presence of the barnase gene decreases the viability of the transfected cells. The pF9A CMV *hRluc*-neo 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 (Cat.# C8640 and C8820). For more information, see the *Flexi[®] Vector Systems Technical Manual #TM254*.

Concentration: 100ng/µl.

GenBank[®] Accession Number: DQ871024

Storage Buffer: The pF9A CMV *hRluc*-neo Flexi[®] Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 8.0).

Storage Conditions: See the Product Information Label for storage recommendations and expiration date. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

Usage Note: 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 pF9A CMV *hRluc*-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 Pml 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 Pml, SgfI and BglII.

Signed by:

R. Wheeler, Quality Assurance

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^(b)Patent Pending.

^(c)U.S. Pat. No. 7,906,282 and European Pat. No. 1341808.

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

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Features List and Map for the pF9A CMV *hRluc*-neo Flexi® Vector

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	1903–2321
<i>Renilla</i> luciferase/neomycin phosphotransferase coding region	2366–4141
synthetic polyadenylation signal	4205–4253
β-lactamase coding region	4514–5374
ColE1-derived plasmid replication of origin	5529–5565
cer site (site for <i>E. coli</i> XerCD recombinase)	6236–6521

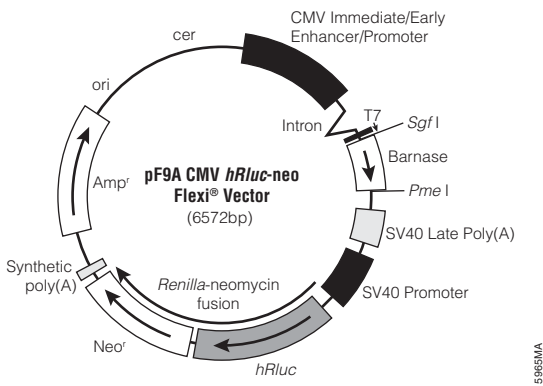


Figure 1. pF9A CMV *hRluc*-neo Flexi® Vector map.

Sequence information and restriction enzyme tables for the Flexi® Vectors are available online at: www.promega.com/vectors/

Additional information is available in Technical Manual #TM254, available online at: www.promega.com/protocols/ or by request.