

## Product Information

### pFLAG-CMV™-3 Expression Vector

Catalog Number **E6783**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

#### Product Description

The pFLAG-CMV-3 Expression Vector is a 6.3 kb vector used for transient or stable expression in mammalian cells. The vector is a derivative of pCMV5<sup>1</sup> used for transient or stable expression and secretion of a properly inserted open reading frame as an N-terminal FLAG® fusion protein.

The promoter-regulatory region of the human cytomegalovirus<sup>2</sup> drives transcription of FLAG-fusion constructs. The preprotrypsin leader sequence<sup>3</sup> precedes the FLAG sequence and directs secretion of the fusion protein into the culture medium. The aminoglycoside phosphotransferase II gene<sup>4</sup> (neo<sup>r</sup>) confers resistance to aminoglycosides such as G 418,<sup>5</sup> allowing for selection of stable transfectants.

The pFLAG-CMV-3 Expression Vector is a shuttle vector containing both bacterial and SV40 origins of replication for propagation in both *Escherichia coli* and mammalian cells. Efficiency of replication and genomic integration is optimal when using host cells that express the SV40 large T antigen (e.g. COS-7). pFLAG-CMV-3 has been used for stable transfection of HEK 293 cells<sup>6</sup>.

The FLAG epitope is a small, hydrophilic 8 amino acid tag (DYKDDDDK)<sup>7</sup> that provides for sensitive detection and high quality purification using ANTI-FLAG® products (visit [www.sigma-aldrich.com](http://www.sigma-aldrich.com) for a complete listing). Removal of the N-terminal FLAG tag is possible using enterokinase, which cleaves following the Asp-Asp-Asp-Asp-Lys recognition site at the C-terminal end of the FLAG peptide.

The pFLAG-CMV-3-BAP Control Plasmid is a 7.7 kb derivative of pCMV5<sup>1</sup> used for transient expression and secretion of N-terminal FLAG bacterial alkaline phosphatase fusion protein in mammalian cells.

The promoter-regulatory region of the human cytomegalovirus<sup>2</sup> drives transcription of bacterial alkaline phosphatase. The preprotrypsin leader sequence<sup>3</sup> precedes the FLAG sequence. The aminoglycoside phosphotransferase II gene<sup>4</sup> (Neo) confers resistance to aminoglycosides such as G 418.<sup>5</sup>

pFLAG-CMV-3-BAP Control Plasmid is a shuttle vector for *E. coli* and mammalian cells. Efficiency of replication and genomic integration is optimal when using an SV40 T antigen-expressing host, such as COS cells.

#### Map positions of key features in the pFLAG-CMV-3 Expression Vector and the pFLAG-CMV-3-BAP Control Plasmid can be found at

[www.sigma.com/vectormaps](http://www.sigma.com/vectormaps).

#### Components

- pFLAG-CMV-3 Expression Vector 20 µg  
Catalog Number E8770  
Supplied as 0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.
- pFLAG-CMV-3-BAP Control Plasmid 20 µg  
Catalog Number C3972  
Supplied as 0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### Storage/Stability

Store at  $-20\text{ }^{\circ}\text{C}$

#### References

1. Andersson, S., *et al.*, Cloning, structure, and expression of the mitochondrial cytochrome P-450 sterol 26-hydroxylase, a bile acid biosynthetic enzyme. *J. Biol. Chem.*, **264**, 8222-8229 (1989).
2. Thomsen, D. R., *et al.*, Cloning, structure, and expression of the mitochondrial cytochrome P-450 sterol 26-hydroxylase, a bile acid biosynthetic enzyme. *Proc. Natl. Acad. Sci. USA*, **81**, 659-663 (1984).
3. Stevenson, B. J., *et al.*, Sequence organization and transcriptional regulation of the mouse elastase II and trypsin genes. *Nucleic Acids Res.*, **21**, 8307-8330 (1986).

4. Brewer, C. B., Cytomegalovirus plasmid vectors for permanent lines of polarized epithelial cells. *Methods in Cell Biology*, **43**, 233-245 (1994).
5. Jimenez, A. and Davies, J., Expression of a transposable antibiotic resistance element in *Saccharomyces*. *Nature*, **287**, 869-871 (1980).
6. Soto, H., *et al.*, The CC chemokine 6Ckine binds the CXC chemokine receptor CXCR3. *Proc. Natl. Acad. Sci. USA*, **95**, 8205-8210 (1998).
7. Hopp, T. P., *et al.*, A short polypeptide marker sequence useful for recombinant protein identification and purification., *Bio/Technology*, **6**, 1204-1210 (1988).

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