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Product Information

pCMV-BICEP™-4 Expression Vector

Catalog Number **E5905**
Storage temperature –20 °C

TECHNICAL BULLETIN

Product Description

pCMV-BICEP-4 is a 5363 bp mammalian expression vector used for transient co-expression of two genes of interest resulting in production of N-terminal FLAG® epitope tagged fusion proteins and N-terminal *c-myc* epitope tagged fusion proteins in mammalian cells. The vector, a derivative of the pCMV5 transient expression vector,[1] contains the internal ribosome entry site (IRES) derived from the encephalomyo-carditis virus (EMCV), allowing translation of two genes from one bicistronic mRNA. The presence of two multiple cloning sites provides for insertion of two independent open reading frames (ORFs). Following transfection into mammalian cells, expression of both ORF1 and ORF2 sequences allows the study of multi-subunit proteins and protein:protein interaction candidates through the capture and detection of fusion-tagged complexes via the FLAG and/or the *c-myc* epitopes. The pCMV-BICEP-4+p53/LTA Control Vector contains a model protein-protein interaction pair, the p53 gene, inserted into MCS1, and the SV40 Large T Antigen, inserted into MCS2.

The promoter-regulatory region of the human cytomegalovirus immediate early promoter[2,3] directs high level transcription of the bicistroninc FLAG fusion and *c-myc* fusion sequences. While translation of the upstream FLAG fusion sequences occurs via normal cap-dependent translational processes, the EMCV IRES[4,5] region controls translation of the downstream *c-myc* fusion by recruiting the ribosomal subunits for cap-independent translational initiation. The pCMV-BICEP-4 expression vector is a shuttle vector containing both bacterial and SV40 origins of replication for propagation in both *E.coli* and mammalian cells. Efficiency of replication in mammalian cells is optimal when using host cells that express the SV40 Large T antigen (e.g. COS-7).

The N-terminal FLAG fusion protein may be detected using the Anti-FLAG® M2 monoclonal antibody (Product No. F 3165) and purified using the Anti-FLAG M2 Agarose (Product No. A 2220). The N-terminal *c-myc* fusion may be detected using the Anti-*c-myc* monoclonal antibody (Product No. M 4439). Sigma-Aldrich offers a wide selection of related Anti-FLAG and Anti-*c-myc* products. Please visit www.sigma-aldrich.com for a complete listing of antibody conjugates, resins, and affinity capture plates.

The following table provides map positions of the features in the pCMV-BICEP-4 Expression Vector. Sequence verification of MCS1 can be performed using the N-CMV-30 primer (Product No. P 5350) while sequence verification of MCS2 can be performed using the C-CMV-24 primer (Product No. P 5475).

pCMV-BICEP™-4 Features

Feature	Map Position
CMV Promoter	166-916
N-CMV-30 Sequencing Primer Binding Site	825-854
FLAG	935-958
MCS1	959-996
IRES	1024-1604
c-myc	1637-1666
MCS2	1667-1701
HGH polyA	1707-2326
C-CMV-24 Sequencing Primer Binding Site	1764-1787
SV40 ori	2345-2689
pBR322 ori	3592-3711
Amp	3858-4776
f1 ori	4911-5363

Reagents

- pCMV-BICEP-4 Expression Vector, 20 µg, E3780, 0.5 mg/ml in 10 mMTris-HCl, pH 8.0, 1 mM EDTA.

- pCMV-BICEP-4+p53/LTA Control Vector, 20 µg, C6864, 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

This product ships on dry ice and storage at –20 °C is recommended.

References

1. Andersson, S., et al., J. Biol. Chem., **264**, 8222-8229 (1989).
2. Thomsen, D. R., et al., Proc. Natl. Acad. Sci. U.S.A., **81**, 659-663 (1984).
3. Chapman, B. S., et al., Nucleic Acids Res., **19**, 3979-3986 (1991).
4. Jang, S. K., et al., J. Virol., **62**, 2636-2643 (1988).
5. Jackson, R. J., et al., Trends Biochem. Sci., **15**, 477-483 (1990).

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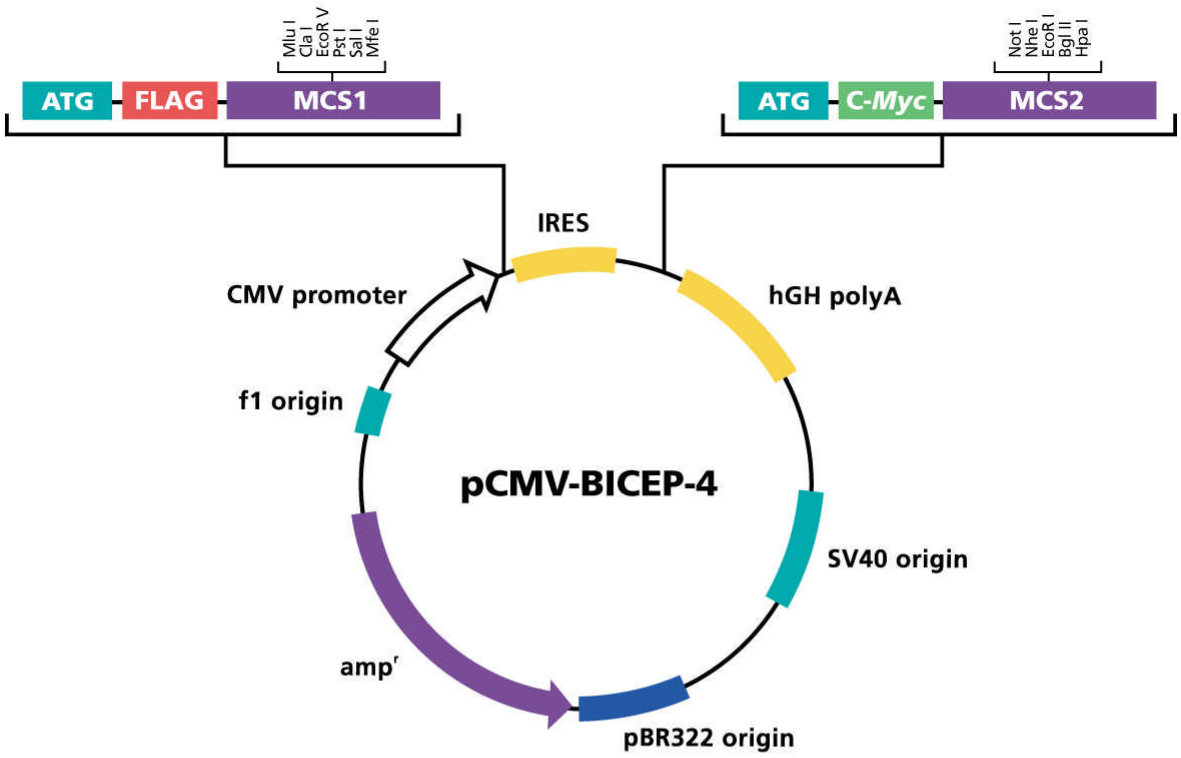
pCMV-BICEP-4 (5.4 kb)



Multiple Cloning Site 1
(pCMV-BICEP-4)

FLAG Peptide Sequence

Met Asp Tyr Lys Asp Asp Asp Asp Lys

Cla I EcoR V Pst I Sal I Mfe I

ATG GAC TAC AAA GAC GAT GAC GAC AAG ACG CGT ATC GAT GAT ATC CTG CAG AGT CGA CAC AAT TGC GCG

TAC CTG ATG TTT CTG CTA CTG CTG TTC TGC GCA TAG CTA CTA TAG GAC GTC TCA GCT GTG TTA ACG CGC

Mlu I

Multiple Cloning Site 2

C-*Myc* Sequence

Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu

Not I Nhe I EcoR I Bgl II Hpa I

ATG GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG GCG GCC GCA GCT AGC GAA TTC GTA GAT CTG TTA ACT

TAC CTT GTT TTT GAG TAG AGT CTT CTC CTA GAC CGC CGG CGT CGA TCG CTT AAG CAT CTA GAC AAT TGA