Technology
Biomyx
Innovation & Convenience for Life Sciences

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pMEV-2HA

Catalog# P1001
Lot#

Materials Provided

1. pMEV-2HA(a): 20μg in 40μl TE (pH7.5), 0.5 mg/ml.
2. pMEV-2HA(b): 20μg in 40μl TE (pH7.5), 0.5 mg/ml.
3. pMEV-2HA(c): 20μg in 40μl TE (pH7.5), 0.5 mg/ml.
4. Product Information Sheet.

Receiving and Storage:

Upon receiving, spin the vials briefly in a microcentrifuge to collect the contents. Store the products at 2-8^{0}C if used immediately and store at –20^{0}C for extended storage.

Prokaryotic/Eukaryotic selection:

Kan/Neo (2228-3022): Confers kanamycin resistance in bacteria; It confers G418 resistance in mammalian cell.

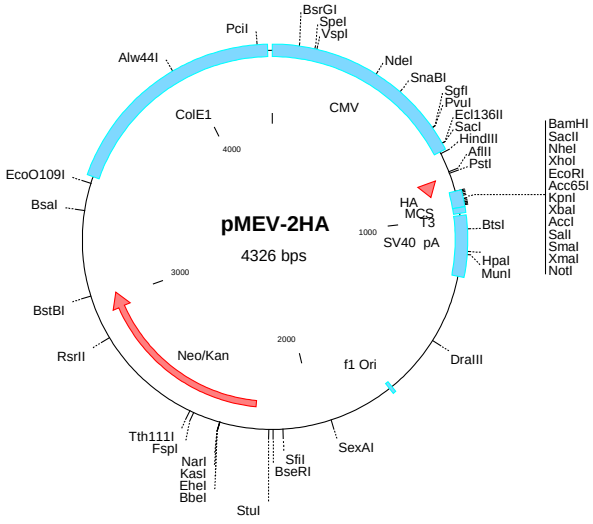
Other Features:

Name	Start	End
CMV Promoter:	1	750
2xHA Coding sequence:	843	902
Multiple Cloning Site (MCS):	903	964
T3 RNA Polymerase Promoter:	964	985
SV40 Polyadenylation signal:	993	1215
f1 Replication Origin:	1691	1703
Neo/Kan-Resistance Gene:	2228	3022
ColE1 Replication Origin:	3478	4310

Maintenance of pMEV-2HA vectors

For help with general DNA manipulation techniques like restriction digestion, ligation and transformation, see *Molecular Cloning: A Laboratory Manual (*Sambrook *et al., 1989*) or *Current Protocols in Molecular Biology (*Ausubel *et al., 1994).* We recommend that you propagate the vectors in *E. coli* strains that are recombinant deficient (*recA*) and endonuclease A-deficient (*endA*) (e.g. DH5α or XL1-Blue).

Circular Map of pMEV-2HA:



About pMEV-2HA vectors

pMEV-2HA vectors are designed for high level protein expression and detection in mammalian cells. The human cytomegalovirus immediate-early (CMV) promoter provides high-level expression in a wide range of mammalian cells. To facilitate the purification and detection of the protein of interest, pMEV-2HA vectors use a 9-aa peptide derived from hemagglutinin influenza virus (aa 114-122) as the affinity tag. When a coding sequence for the protein of interest is inserted in the MCS of pMEV-2HA, in frame with the HA-encoding sequence, the resulting vector expresses a fusion protein with the HA tag (MGYPYDVPDYAYPYDVPDYA) at the N-terminus that can be easily detected or used in immunoprecipitation with anti HA antibody. The use of 2 x HA tag increases the sensitivity of anti-HA antibody-based detections. In addition, the vectors are only 4.3 kilo-base pairs in size for easy cloning and high plasmid purification yields owing to the use of the Neo/Kan coding sequence in the vectors for both bacterial selection with kanamycin and mammalian cell selection with G418. Stable mammalian cell lines can be established by selecting clones with G418 after transfection with pMEV vectors.

Cloning into pMEV-2HA vectors

The pMEV-2HA vectors are fusion vectors. To ensure proper protein expression, gene of interest should be cloned in frame with the initiation ATG of HA-encoding sequence (base pairs 843-845, see multiple cloning site sequences for details). The resulting clone will express the gene of interest fused with 2XHA expression tag at its N-terminus, allowing protein detection or purification with anti-HA antibody (not supplied).

Multiple cloning site of pMEV-2HA frame A

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843                          2xHA (YPYDVPDYA)
ATGGGATACCCTTACGACGTTCCTGATTACGCTTACCCTTACGACGTTCCTGATTACGCT
MetGlyTyrProTyrAspValProAspTyrAlaTyrProTyrAspValProAspTyrAla

                    Xho I                    Xba I      Xma I
BamH I    Nhe I     EcoR I        Kpn I        Sal I      Not I
GGATCCGCGGCTAGCCTCGAGAATTCACGCGTGGTACCTCTAGAGTCGACCCGGGCGGCC
GlySerAlaAlaSerLeuGluAsnSerArgValValProLeuGluSerThrArgAlaAla

GCTTCCCTTTAGTGAGGGTTAATGCTTC
AlaSerLeu***
```

Multiple cloning site of pMEV-2HA frame B

```
                    Xho I                    Xba I      Xma I
  BamH I    Nhe I     EcoR I        Kpn I        Sal I      Not I
atGGATCCGCGGCTAGCCTCGAGAATTCACGCGTGGTACCTCTAGAGTCGACCCGGGCGG
MetAspProArgLeuAlaSerArgIleHisAlaTrpTyrLeu***
```

Multiple cloning site of pMEV-2HA frame C

```
                    Xho I                    Xba I      Xma I
  BamH I              EcoR I        Kpn I        Sal I      Not I
tGGATCCGCGGCTAGCCTCGAGAATTCACGCGTGGTACCTCTAGAGTCGACCCGGGCGG
TrpIleArgGlyLysProArgGluPheThrArgGlyThrSerArgValAspProGlyGl
```

Note: *Nucleotides 903-904 (a and t in lower cases before BamHI) are inserted in frame B to adjust the reading frame.*

General References:

Sambrook, J. *et al,* 1989, **Molecular Cloning: A Laboratory manual**. Second Edition. (Plainview, New York: Cold Spring Harbor Laboratory Press).

Ausubel F. M. *et al*, 1994, **Current Protocols in Molecular Biology** (New York: Greene Publishing Associates and Wiley-Interscience).