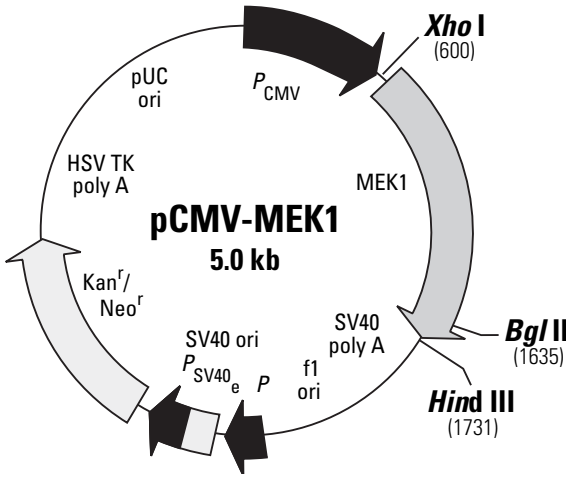
Vector Information

pCMV-MEK1 Vector Information PT3384-5

GenBank Accession No.: Submission in progress. Sold as part of Cat. No. 631927



Restriction Map of pCMV-MEK1. All restriction sites shown are unique.

Description & Use:

pCMV-MEK1 is designed for the constitutive expression of MAPK/ERK kinase 1 (MEK 1) in mammalian cells. It can also be used to study the effects of a given stimulus on the MAPK signaling pathway. In conjunction with Clontech's *In Vivo* Kinase Assay Kits, pCMV-MEK1 is an ideal positive control for studying a gene or molecule and its affect on kinase activation in the MAPK pathway. MEK1, a Raf isoform, phosphorylates and activates ERK-1 and ERK-2 (1–3). Studies show that ERK1/2 are activated by mitogens in most cell types, linking their involvement in mitogenic signaling (2). ERKs may also be linked to cell transformation, differentiation, and apoptosis in some cell types (*ibid.*).

pCMV-MEK1 contains the gene encoding MEK1 (human), which is controlled by the human cytomegalovirus (CMV) promoter. SV40 polyadenylation signals downstream of MEK1 direct proper processing of the 3' end of the mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40T-antigen. A neomycin resistance cassette (Neo^r)—consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene—allows stably transfected eukaryotic cells to be selected using G418 (4). A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pCMV-MEK1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production. The recombinant vector can be transfected into mammalian cells using any standard method.

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Location of features:

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- MAPK/ERK kinase 1 (MEK1) gene:
  Start codon (ATG): 608–610; stop codon: 1727–1729
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1928–1933 & 1957–1962; mRNA 3' ends: 1966 & 1978
- f1 single-strand DNA origin: 2025–2480 (Packages the noncoding strand of MEK1.)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2542–2547; –10 region: 2565–2570
  Transcription start point: 2577
- SV40 origin of replication: 2821–2956
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2654–2725 & 2726–2797
  21-bp repeats: 2801–2821, 2822–2842 & 2844–2864
  Early promoter element: 2877–2883
  Major transcription start points: 2873, 2911, 2917 & 2922
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 3005–3007; stop codon: 3797–3799
  G→A mutation to remove *Pst* I site: 3187
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3533
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 4035–4040 & 4048–4053
- pUC plasmid replication origin: 4384–5027

Propagation in *E. coli:*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Brott, B. K., *et al.* (1993) *Cell Growth Differ.* **4**:921–929.
2. Robinson, M. J. & Cobb, M. H. (1997) *Curr. Opin. Biol.* **9**:180–186.
3. Cobb, M. H. & Goldsmith, E. J. (1995) *J. Biol. Chem.* **270**:14843–14846.
4. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.