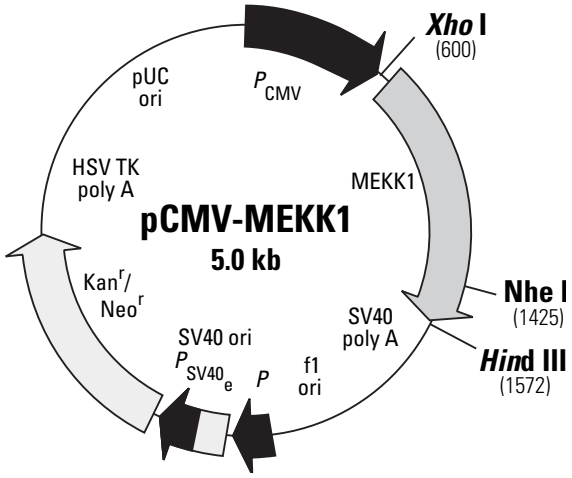
Vector Information

pCMV-MEKK1 Vector Information PT3385-5

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



Restriction Map of pCMV-MEKK1. All restriction sites shown are unique.

Description & Use:

pCMV-MEKK1 is designed for the constitutive expression of MEK Kinase 1 (MEKK1) in mammalian cells. It can also be used to study the effects of a given stimulus on the JNK/SAPK pathway. In conjunction with Clontech's *In Vivo* Kinase Assay Kits, pCMV-MEKK1 is an ideal positive control for studying a gene or molecule and its affect on kinase activation in the JNK/SAPK pathway.

MEKK1 mediates cellular responses to diverse stimuli, such as cytokines, growth factors, and other stressors via activation of JNK/SAPK signaling pathway. It has also been reported that MEKK1 is a possible regulator in signal transduction pathways involving apoptosis (1). To a lesser extent, MEKK1 can also activate the ERK pathway via phosphorylation of MEK (2, 3); however, it is more efficient in activating the JNK/SAPK than ERK (4, 5).

pCMV-MEKK1 contains a gene encoding MEKK1 (mouse) that is driven by the human cytomegalovirus (CMV) promoter. SV40 polyadenylation signals downstream of the MEKK1 gene 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 SV40 T-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 (6). A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pCMV-MEKK1 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
- MEK kinase 1 (MEKK1) gene:
  Start codon (ATG): 608–610; stop codon: 1568–1570
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1769–1774 & 1798–1803; mRNA 3′ ends: 1807 & 1819
- f1 single-strand DNA origin: 1866–2321 (Packages the noncoding strand of MEKK1.)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2383–2388; –10 region: 2406–2411
  Transcription start point: 2418
- SV40 origin of replication: 2662–2797
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2495–2566 & 2567–2638
  21-bp repeats: 2642–2662, 2663–2683 & 2685–2705
  Early promoter element: 2718–2724
  Major transcription start points: 2714, 2752, 2758 & 2763
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2846–2848; stop codon: 3638–3640
  G→A mutation to remove *Pst* I site: 3028
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3374
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3876–3881 & 3889–3894
- pUC plasmid replication origin: 4225–4868

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:

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3. Robinson, M. J. & Cobb, M. H. (1997) *Curr. Opin. Cell Biol.* **9**:180–186.
4. Minden, A., *et al.* (1994) *Science* **266**:1719–1723.
5. Lin, A., *et al.* (1995) *Science* **268**:286–290.
6. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

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