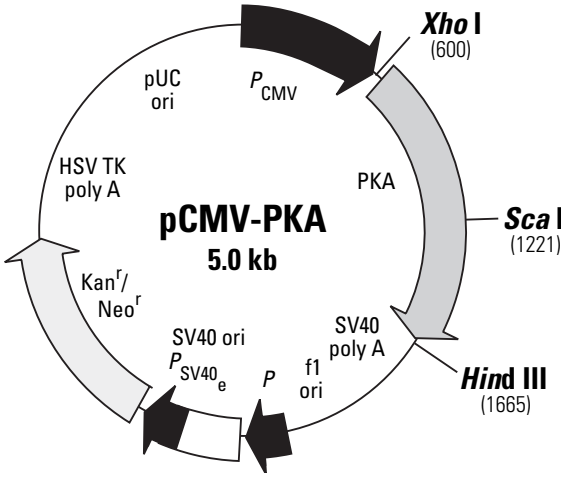
Vector Information

pCMV-PKA Vector Information PT3386-5

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



Restriction Map of pCMV-PKA. All sites shown are unique.

Description & Use:

pCMV-PKA is designed for the constitutive expression of Protein Kinase A (PKA) (mouse) in mammalian cells. It can also be used to study the effects of a given stimulus on the PKA signaling pathway. In conjunction with Clontech's *In Vivo* Kinase Assay Kits, pCMV-PKA is an ideal positive control for studying a gene or molecule and its affect on kinase activation in the PKA pathway. cAMP, a ubiquitous second messenger, regulates expression of cellular functions including metabolism, cell proliferation, and neuronal signaling via activation of the PKA-dependent phosphorylation of the CREB transcription factor (1–3). pCMV-PKA contains the gene encoding the catalytic subunit of PKA, which is driven by the human cytomegalovirus (CMV) promoter.

SV40 polyadenylation signals downstream of the PKA 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 SV40T-antigen. A neomycin resistance cassette (Neo^r)—consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene ofTn5, 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-PKA 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
- Protein Kinase A (PKA) gene
  Start codon (ATG): 608–610; Stop codon: 1661–1663
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1862–1867 & 1891–1896; mRNA 3′ ends: 1900 & 1912
- f1 single-strand DNA origin: 1959–2414 (Packages the noncoding strand of PKA.)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2476–2481; –10 region: 2499–2504
  Transcription start point: 2511
- SV40 origin of replication: 2755–2890
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2588–2659 & 2660–2731
  21-bp repeats: 2735–2755, 2756–2776 & 2778–2798
  Early promoter element: 2811–2817
  Major transcription start points: 2807, 2845, 2851 & 2856
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2939–2941; stop codon: 3731–3733
  G→A mutation to remove *Pst* I site: 3121
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3467
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3969–3974 & 3982–3987
- pUC plasmid replication origin: 4318–4961

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. Montminy, M. (1997) *Annu Rev. Biochem.* **66**:807–822.
2. Hagiwara, M., *et al.* (1993) *Mol. Cell Biol.* **13**:4852–4859.
3. Lee, K. A. (1991) *Curr Opin. Cell Biol.* **3**:953–959.
4. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.