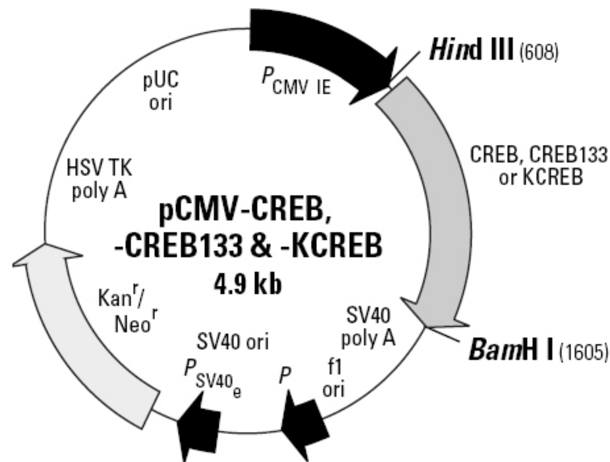
CREB Dominant-Negative Vector Set Information

PT3457-5

Cat. No. 631925



Combined map of pCMV-CREB, pCMV-CREB133 & pCMV-KCREB. All restriction sites shown are unique. The CREB Dominant-Negative Vector Set includes three vectors; each vector contains a different CREB coding sequence, as shown above.

Description

CREB (CRE-binding protein) is a member of the leucine zipper family of transcription factors and forms both a homodimer with itself and heterodimers with other leucine zipper proteins (1, 2). CREB also has a kinase-inducible domain, which contains consensus phosphorylation sites for several kinases, such as protein kinase A (1, 2). The CREB Dominant-Negative Vector Set consists of three vectors:

- **pCMV-CREB Vector**—constitutively expresses the human wild-type (wt) CREB protein.
- **pCMV-CREB133 Vector**—expresses a mutant variant of the human CREB protein that contains a serine to alanine mutation corresponding to amino acid 133 in the mutant mouse CREB protein. This mutation blocks phosphorylation of CREB, thus preventing transcription.
- **pCMV-KCREB Vector**—expresses a mutant variant of the human CREB protein that contains mutations in its DNA-binding domain. KCREB acts as a dominant repressor by forming an inactive dimer with CREB, blocking its ability to bind cAMP-regulated enhancer element (CRE).

These proteins are expressed at high levels from the constitutive CMV promoter. The SV40 polyadenylation sequence directs proper processing of the 3' end of the mRNAs. The vector backbone 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 Tn5 neomycin/kanamycin resistance gene, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene—allows kanamycin selection in *E. coli* and neomycin selection in eukaryotic cells. The vector backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

The CREB Dominant-Negative Vector Set can be used to monitor signal transduction pathways related to CREB. In conjunction with reporter systems (such as our *cis*-acting reporter vectors), you can monitor CREB-induced transcription by assaying for the reporter. For example, CREB activation can be measured in cells cotransfected with pCMV-CREB and pCRE-d2EGFP (Cat. No. 631802) by treating the transfected cells with forskolin and observing EGFP expression by FACS analysis or fluorescence microscopy. Cells expressing dominant-negative CREB133 or KCREB will not respond to this stimulus, so induced transcription by forskolin is inhibited. For more information about Pathway Profiling Vectors, visit our web site at **www.clontech.com**.

These vectors can be transfected into mammalian cells using any standard method. Stable transformants can be selected using G418 (3).

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Note: The following list of features is based on the pCMV-CREB Vector. Due to different subcloning parameters, the pCMV-CREB133 Vector and pCMV-KCREB differ from pCMV-CREB by a few base pairs. Complete sequence and restriction digest information for all of these vectors are available at **vectors.clontech.com.**

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
- CREB variant (each vector contains one of the following):
  - **wild-type CREB only:** Start codon (ATG): 620–622; stop codon: 1601–1603
  - **CREB133 only:** Start codon (ATG): 613–615; stop codon: 1593–1595
  - T→G (Ser-to-Ala) mutation: 967
  - **KCREB only:** Start codon (ATG): 613–615; stop codon: 1603–1605
  - G→T (Arg-to-Leu) mutations: 1479 & 1482; addition of Lys (AAG): 1487–1489
- SV40 early mRNA polyadenylation signal:
  - Polyadenylation signals: 1764–1768 & 1793–1797; mRNA 3' ends: 1802 & 1814
- f1 single-strand DNA origin: 1861–2316 (Packages the noncoding strand of CREB.)
- Bacterial promoter for expression of Kan^r gene:
  - –35 region: 2378–2383; –10 region: 2401–2406
  - Transcription start point: 2413
- SV40 origin of replication: 2657–2734
- SV40 early promoter:
  - Enhancer (72-bp tandem repeats): 2490–2561 & 2562–2635
  - 21-bp repeats: 2637–2657, 2658–2678 & 2680–2700
  - Early promoter element: 2713–2719
  - Major transcription start points: 2709, 2747, 2753 & 2758
- Kanamycin/neomycin resistance gene:
  - Neomycin phosphotransferase coding sequences: start codon (ATG): 2841–2843; stop codon: 2633–3635
  - G→A mutation to remove *Pst* I site: 3023
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3369
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal:
  - Polyadenylation signals: 3871–3876 & 3884–3889
- pUC plasmid replication origin: 4225–4863

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 JM101 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. Walton, K. M., *et al.* (1992) *Mol. Endrocrinol.* **92**:647–655.
2. Jean, D., *et al.* (1995) *J. Biol. Chem.* **273**:24884–24890.
3. Gorman, C. (1985) In *DNA cloning: A practical approach, Vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, UK) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

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