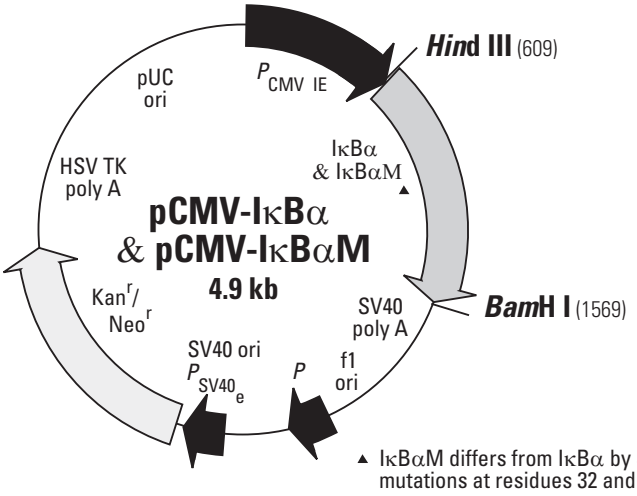
Vector Information

IκBα Dominant-Negative Vector Set Information

PT3429-5

Cat. No. 631923



Map of pCMV-IκBα and pCMV-IκBαM Vectors. All restriction sites shown are unique.

Description

The IκBα Dominant-Negative Vector Set consists of two vectors, pCMV-IκBα and pCMV-IκBαM. These vectors are convenient tools for examining NFκB regulation by manipulating its inhibitor, IκBα. In uninduced cells, IκBα binds NFκB and inhibits its activation by preventing NFκB from translocating to the nucleus. However, upon activation of NFκB by agents like TNF, IκBα can be phosphorylated, thus leading to the disassociation of IκBα from NFκB. pCMV-IκBαM contains two mutations that prevent this phosphorylation step; therefore, cells expressing IκBαM block the NFκB pathway (1–3). The IκBα gene and IκBαM gene differ by serine to alanine mutations at residues 32 and 36 (1). Both 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 SV40T antigen. A neomycin-resistance cassette (Neor)—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

pCMV-IκBα can be used to screen drug candidates for their effects on NFκB pathway or to study the involvement of upstream kinases which precede IκBα degradation. IκBα overexpression eliminates any low-level stimulation produced from the culture medium, and ensures that any NFκB stimulation measured is due to the agent you are testing. pCMV-IκBαM can be used to "knock down" expression of endogenous IκBα or block NFκB signaling in a particular cell line.

In conjunction with one of our NFκB *cis*-acting reporter vectors, such as pNFκB-SEAP, pNFκB-Luc, or pNFκB-d2EGFP (Cat. Nos. 631905, 631904, and 631803, resp.), you can measure the activation of NFκB in your system by measuring the expression of the reporter gene (4). For more information about our Signal Transduction Vectors, visit our web site at www.clontech.com

Both vectors can be transfected into mammalian cells using any standard method. Stable transformants can be selected using G418 (5).

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Note: The following list of features is based on the pCMV-IκBα Vector. pCMV-IκBαM differs from pCMV-IκBα by two mutations at residues 32 & 36 of IκBα. Due to different subcloning parameters, the pCMV-IκBαM Vector is 30 bp smaller than pCMV-IκBα.

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
- IκBα gene: Start codon (ATG): 613–615; stop codon: 1564–1566
- IκBαM only: Start codon (ATG): 584–586; stop codon: 1535–1537
  AG→GC (Ser→Ala) mutation: 677 & 678; T→G (Ser→Ala) mutation: 689
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1727–1731 & 1756–1760; mRNA 3' ends: 1765 & 1777
- f1 single-strand DNA origin: 1824–2279 (Packages the noncoding strand of IκBα or IκBαM.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2341–2346; –10 region: 2364–2369
  Transcription start point: 2376
- SV40 origin of replication: 2620–2697
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2453–2524 & 2525–2598
  21-bp repeats: 2600–2620, 2621–2641 & 2643–2663
  Early promoter element: 2676–2682
  Major transcription start points: 2672, 2710, 2716 & 2721
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: start codon (ATG): 2804–2806; stop codon: 3596–3598
  G→A mutation to remove *Pst* I site: 2986
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3332
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3834–3839 & 3847–3852
- pUC plasmid replication origin: 4188–4826

*All numbers are correct for pCMV-IκBα. For pCMV-IκBαM, add 1 bp for all sequence numbers after nucleotide number 593.

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. Feig, B., *et al.* (1999) *Surgery* 126:399–405.
2. Brown, K., *et al.* (1995) *Science* 267:1485–1491.
3. Chen, Z. J., *et al.* (1995) *Genes Devel.* 9:1586–1597.
4. Mercury IκBα Dominant-Negative Vector Set (January 2000) *Clontechniques* XV(1):18–19.
5. 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.