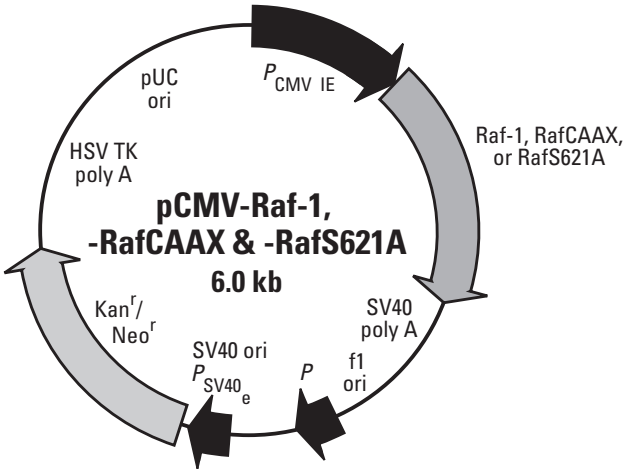
Vector Information

Raf Dominant-Negative Vector Set Information PT3474-5

Catalog No. 631926



Combined map of pCMV-Raf-1, pCMV-RafCAAX & pCMV-RafS621A Vectors. The Raf Dominant-Negative Vector Set includes three vectors; each vector contains a different Raf coding sequence.

Description

Raf-1, a serine/threonine protein kinase, acts as an intermediate link between upstream and downstream kinases in response to various growth factors and mitogens (1, 2). The activation of Raf-MAP (mitogen-activated protein) kinase cascade is a critical step in cellular transformation induced by oncogenic Ras (3, 4); however, the mechanisms by which Ras mediates Raf-1 activation is unclear. The Raf Dominant-Negative Vector Set is a convenient tool for studying mechanisms that affect Raf-1 regulation. The set consists of three vectors:

- **pCMV-Raf-1 Vector**—expresses the human, wild-type (wt) v-Raf-1 protein.
- **pCMV-RafCAAX Vector**—expresses a constitutively active form of the Raf protein. This vector encodes K-ras carboxyl-terminal localization signals, which are targeted to the plasma membrane when RafCAAX is expressed in cells. Studies have shown that Ras activation of Raf involves the recruitment of Raf to the plasma membrane where a separate Ras-independent activation of Raf occurs (5–7). Thus, when RafCAAX is expressed in cells, it is enzymatically active, independent of Ras activation.
- **pCMV-RafS621A Vector**—expresses a dominant-negative form of the Raf protein that blocks Raf pathway activation. RafS621A protein contains a serine-to-alanine mutation at amino acid 621, altering the phosphorylation site for Raf activation, and therefore, blocking phosphorylation and activation of Raf (2).

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

These vectors can be transfected into mammalian cells using any standard method. Stable transformants can be selected using G418 (8).

The Raf Dominant-Negative Vector Set can be used with our *cis*-acting reporter vectors, such as pSRE-SEAP (Cat. No. 631901); this combination allows you to set up a complete assay system to measure differences in activation of the Raf-1 pathway.

(PR59992; published 29 September 2005)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Note: The following list of features is based on the pCMV-Raf-1 Vector. pCMV-RafS621A differs from pCMV-Raf-1 by a single point mutation. However, pCMV-RafCAAX Vector is 68 bp larger than pCMV-Raf-1 and pCMV-RafS621A due to differences in subcloning parameters. Complete sequence and restriction digest information for all of these vectors are available at **orders.clontech.com/clontech/techinfo/vectors/vectorsC/pCMV-Raf.shtml.**

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
- Raf variant (each vector contains one of the following):
  wild-type Raf-1: Start codon (ATG): 620–622; stop codon: 2564–2566
  RafCAAX only: Start codon (ATG): 609–612; stop codon: 2622–2624
  RafS621A only: Start codon (ATG): 620–622; stop codon: 2564–2566
  T→G (Ser-to-Ala) mutation: 2480
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2727–2731 & 2756–2760; mRNA 3' ends: 2765 & 2777
- f1 single-strand DNA origin: 2824–3279 (Packages the noncoding strand of Raf-1.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 3341–3346; –10 region: 3364–3369
  Transcription start point: 3376
- SV40 origin of replication: 3620–3697
- SV40 early promoter:
  Enhancer (72 bp tandem repeats): 3453–3524 & 3525–3598
  21 bp repeats: 3600–3620, 3621–3641 & 3643–3663
  Early promoter element: 3676–3682
  Major transcription start points: 3672, 3710, 3716 & 3721
- Kanamycin/neomycin resistance gene:
  Neomycin phosphotransferase coding sequences: start codon (ATG): 3804–3806; stop codon: 4596–4598
  G→A mutation to remove *Pst* I site: 3986
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 4332
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal:
  Polyadenylation signals: 4834–4839 & 4847–4852
- pUC plasmid replication origin: 5188–5826

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) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Morrison, D. K. & Cutler, R. E. (1997) *Curr. Opin. Cell Biol.* **9**:174–179.
2. Mischak, H., *et al.* (1996) *Mol. Cell Biol.* **16**:5409–5418.
3. Cowley, S., *et al.* (1994) *Cell* **77**:841–852.
4. Khosravi-Far, R., *et al.* (1995) *Mol. Cell Biol.* **13**:6643–6453.
5. Mineo, C., *et al.* (1996) *J. Biol. Chem.* **272**:10345–10348.
6. Leevers, S. J., *et al.* (1994) *Nature* **369**:411–414.
7. Stokoe, D., *et al.* (1994) *Science* **266**:1792–1793.
8. 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.