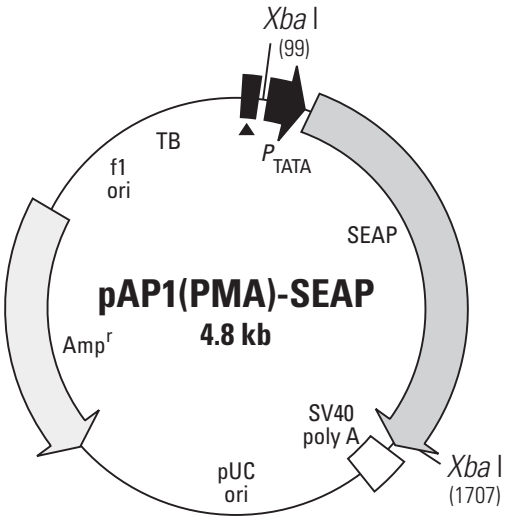
Vector Information

pAP1(PMA)-SEAP† Vector Information PT3351-5

GenBank Accession No.: Submission in progress. Catalog No. 631907



TB=Transcription Blocker
▲ = pAP1(PMA) element

Restriction Map of pAP1(PMA)-SEAP.

Description:

pAP1(PMA)-SEAP is a member of the signal transduction reporter vectors. It is designed for monitoring the induction of the protein kinase C (PKC) signal transduction pathway, as well as related pathways such as the MAPK pathway. pAP1(PMA)-SEAP encodes the secreted alkaline phosphatase (SEAP) reporter gene (1–3). The vector contains six tandem copies of the AP1 enhancer, located upstream of SEAP, that responds specifically to phorbol ester treatment (4). AP1(PMA) is fused to a minimal TA promoter, the TATA box from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. The SEAP coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the SEAP transcript in eukaryotic cells. A synthetic transcription blocker (TB) is located upstream of AP1(PMA), which is composed of adjacent polyadenylation and transcription pause sites for blocking nonspecific transcription (5). The vector backbone also contains an f1 origin for single-stranded DNA production, a pUC origin of replication, and an ampicillin resistance gene for propagation and selection in *E. coli*.

Use:

Activation of the protein kinase C pathway by adding phorbol esters (PMA) results in activator protein 1 binding the AP1 element on the vector and initiating transcription of SEAP. The secreted SEAP enzyme can be assayed directly from the culture medium using one of Clontech's Great EscAPe™ SEAP Detection Kits (Cat. Nos. 631701 & 631704). The pAP1(PMA)-SEAP Vector can be transfected into mammalian cells by any standard method. Stable cell lines expressing this construct can be developed by cotransfecting with a vector containing an antibiotic resistance gene, such as neomycin, hygromycin, or puromycin, and selecting resistant clones.

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Location of features:

- Activator Protein 1 (AP1) element (4): 48–89
- TA minimal promoter: 107–113
- Secreted alkaline phosphatase (SEAP) gene:
  - SEAP coding sequences:
    - start codon (ATG): 185–187; stop codon: 11742–1744
    - signal peptide: 185–235
    - mature protein: 236–1741
    - C-terminal extension to SEAP: 1703–1741
- SV40 late mRNA polyadenylation signal: 1855–1860
  - mRNA 3' end: 1874
- pUC plasmid replication origin: 2253–2896
- Ampicillin resistance gene:
  - Promoter: –35 region: 2974–3969; –10 region: 3951–3946
  - Transcription start point: 3939
  - Ribosome binding site: 3916–3912
  - β-lactamase coding sequences:
    - start codon (ATG): 3904–3902; stop codon: 3046–3044
  - β-lactamase signal peptide: 3904–3836
  - β-lactamase mature protein: 3835–3047
- f1 single-strand DNA origin (packages the noncoding strand of SEAP): 4036–4491
- Transcription blocker (TB): 4622–4775
  - Synthetic polyadenylation site (6): 4622–4670
  - Transcription pause site from human α2 globin gene (7): 4684–4775

Propagation in *E. coli*:

- Suitable host strains: DH5α and other general purpose strains. Single-stranded DNA production requires a host containing an F' episome such as JM109.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

References:

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