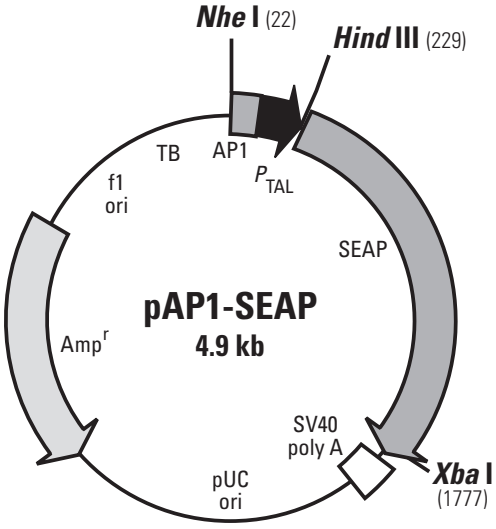
Vector Information

pAP1-SEAP Vector Information PT3279-5

GenBank Accession No.: Submission in progress. Catalog No. 631903



TB=Transcription Blocker

Restriction Map of pAP1-SEAP. All restriction sites are unique.

Description:

pAP1-SEAP is designed for monitoring induction of the activator protein 1 (AP1; 1) and the stress-activated protein kinase/Jun N-terminal kinase (SAPK/JNK) signal transduction pathway (2). pAP1-SEAP contains the secreted alkaline phosphatase (SEAP) reporter gene (3–5). This vector also contains four tandem copies of the AP1 enhancer fused to a TATA-like promoter (P_{TAL}) region from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. After transcription factors bind AP1, transcription is induced and the reporter gene is activated.

The SEAP coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the SEAP transcript in eukaryotic cells. Located upstream of AP1 is a synthetic transcription blocker (TB), which is composed of adjacent polyadenylation and transcription pause sites for reducing background transcription (6). 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:

pAP1-SEAP is designed to measure the binding of transcription factors to AP1, providing a direct measurement of activation for this pathway. For example, the addition of serum or growth factors to the cell-culture medium induces the binding of transcription factors to AP1, which initiates transcription of SEAP. Alternatively, you can cotransfect this vector with an expression vector containing your gene of interest to monitor pathway activation. The secreted SEAP enzyme can be assayed directly from the culture medium using one of Clontech's Great EscAPe Chemiluminescence Detection Kits (Cat. Nos. 631701, 631704). In addition, the SEAP assay permits time-course studies not possible with assays dependent on cell lysates. The pAP1-SEAP Vectors can be transfected into mammalian cells by any standard method. For selecting stable clones, cotransfect with a vector containing an antibiotic resistance gene, such as neomycin, hygromycin, or puromycin.

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Location of features:

- Activator Protein 1 (AP1; 1) element: 27–72
- TATA-like promoter (P_{TAL}): 79–227
- Secreted alkaline phosphatase (SEAP) gene:
  - SEAP coding sequences:
    - start codon (ATG): 255–257; stop codon: 1812–1814
    - signal peptide: 255–305
    - mature protein: 306–1811
    - C-terminal extension to SEAP: 1773–1811
- SV40 late mRNA polyadenylation signal: 1925–1930
  - mRNA 3' end: 1944
- pUC plasmid replication origin: 2323–2966
- Ampicillin resistance gene:
  - Promoter: –35 region: 4044–4039; –10 region: 4021–4016
  - Transcription start point: 4009
  - Ribosome binding site: 3986–3982
  - β-lactamase coding sequences:
    - start codon (ATG): 3974–3972; stop codon: 3116–3114
  - β-lactamase signal peptide: 3974–3906
  - β-lactamase mature protein: 3905–3117
- f1 single-strand DNA origin (packages the noncoding strand of SEAP): 4106–4561
- Transcription blocker (TB): 4692–4845
  - Synthetic polyadenylation site (7): 4692–4740
  - Transcription pause site from human α2 globin gene (8): 4754–4845

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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