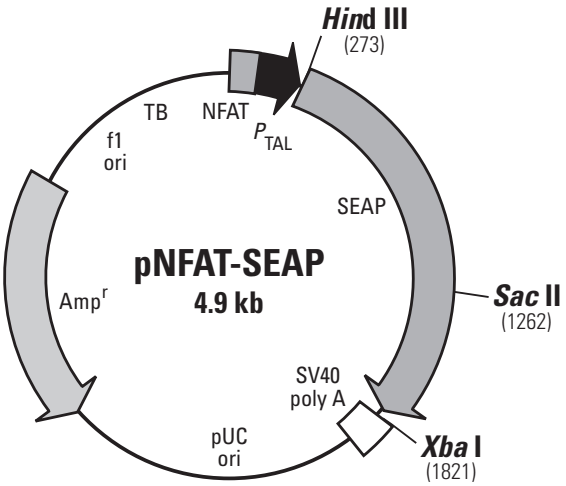
Vector Information

pNFAT-SEAP Vector Information PT3287-5

GenBank Accession No.: Submission in progress. Sold as part of Cat. No. 631910



TB=Transcription Blocker

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

Description:

pNFAT-SEAP is designed to monitor the activation of NFAT and NFAT-mediated signal transduction pathways. Several pathways are associated with the NFAT response element (1), including calcineurin and protein kinase C (PKC; 2). pNFAT-SEAP contains the secreted alkaline phosphatase (SEAP) reporter gene (3–5). This vector also contains three tandem copies of the NFAT consensus sequence fused to a TATA-like promoter (P_{TAL}) region from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. After endogenous NFAT proteins bind the response element, 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 NFAT 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:

pNFAT-SEAP is designed to measure the binding of transcription factors to NFAT, providing a direct measurement of activation for this pathway. For example, treatment of cells with PMA induces the binding of endogenous NFAT proteins to the response element, 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 pNFAT-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:

- NFAT response element (NFAT; 1): 27–116
- TATA-like promoter (P_{TAL}): 123–271
- Secreted alkaline phosphatase (SEAP) gene:
  - SEAP coding sequences:
    - start codon (ATG): 299–301; stop codon: 1856–1858
    - signal peptide: 299–349
    - mature protein: 350–1855
    - C-terminal extension to SEAP: 1817–1855
- SV40 late mRNA polyadenylation signal: 1969–1974
  - mRNA 3' end: 1988
- pUC plasmid replication origin: 2367–3010
- Ampicillin resistance gene:
  - Promoter: –35 region: 4088–4083; –10 region: 4065–4060
  - Transcription start point: 4053
  - Ribosome binding site: 4030–4026
  - β-lactamase coding sequences:
    - start codon (ATG): 4018–4016; stop codon: 3160–3158
  - β-lactamase signal peptide: 4018–3950
  - β-lactamase mature protein: 3949–3161
- f1 single-strand DNA origin (packages the noncoding strand of SEAP): 4150–4605
- Transcription blocker (TB): 4736–4889
  - Synthetic polyadenylation site (7): 4736–4784
  - Transcription pause site from human α2 globin gene (8): 4798–4889

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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