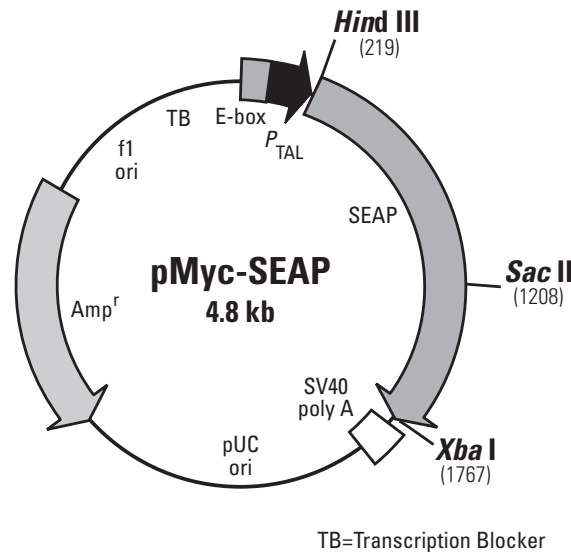


pMyc-SEAP Vector Information

PT3289-5

GenBank Accession No.: Submission in progress.

Sold as part of Cat. No. 631910



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

Description:

pMyc-SEAP is designed to monitor the activation of cMyc and cMyc-mediated signal transduction pathways. Overexpression of the Myc protein causes cell transformation via activation of genes whose products are required for cell growth. The Myc protein forms a heterodimer complex with the Max protein, which binds to the E-box DNA binding element (1) and initiates transcription of genes responsible for cellular proliferation (2). pMyc-SEAP contains the secreted alkaline phosphatase (SEAP) reporter gene (3–5). This vector also contains six tandem copies of the E-box consensus sequence fused to a TATA-like promoter (P_{TAL}) region from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. After cMyc proteins bind E-box, 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 the response element 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:

pMyc-SEAP is designed to measure the binding of transcription factors to E-box, providing a direct measurement of activation for this pathway. For example, the addition of serum or growth factors induces the binding of transcription factors to E-box, 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 permits time-course studies not possible with assays dependent on cell lysates. The pMyc-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:

- cMyc DNA binding element (E-box; 1): 27–62
- TATA-like promoter (P_{TAL}): 69–217
- Secreted alkaline phosphatase (SEAP) gene:
 - SEAP coding sequences:
 - start codon (ATG): 245–247; stop codon: 1802–1804
 - signal peptide: 245–295
 - mature protein: 296–1801
 - C-terminal extension to SEAP: 1763–1801
- SV40 late mRNA polyadenylation signal: 1915–1920
 - mRNA 3' end: 1934
- pUC plasmid replication origin: 2313–2956
- Ampicillin resistance gene:
 - Promoter: –35 region: 4034–4029; –10 region: 4011–4006
 - Transcription start point: 3999
 - Ribosome binding site: 3976–3972
 - β -lactamase coding sequences:
 - start codon (ATG): 3964–3962; stop codon: 3106–3104
 - β -lactamase signal peptide: 3964–3896
 - β -lactamase mature protein: 3895–3107
- f1 single-strand DNA origin (packages the noncoding strand of SEAP): 4096–4551
- Transcription blocker (TB): 4682–4835
 - Synthetic polyadenylation site (7): 4682–4730
 - Transcription pause site from human $\alpha 2$ globin gene (8): 4744–4835

Propagation in *E. coli*:

- Suitable host strains: DH5 α 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 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

References:

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Notice to Purchaser

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.

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