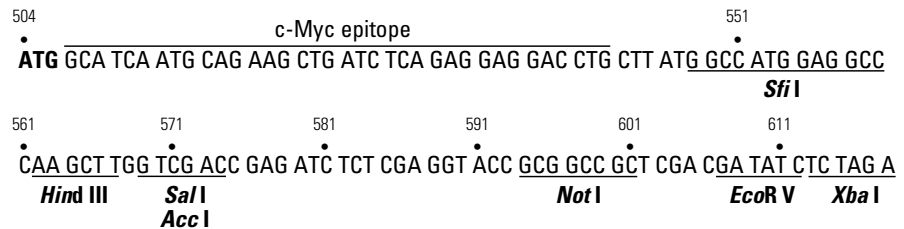
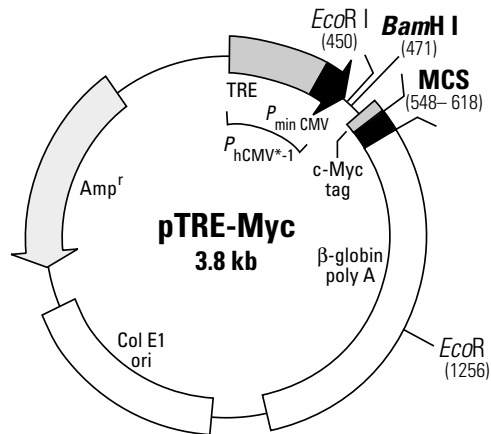


pTRE-Myc Vector Information

GenBank Accession #: Submission in progress.

PT3398-5

Catalog #6247-1



Map and Multiple Cloning Site (MCS) of pTRE-Myc Vector. Unique restriction sites are in bold.

Description:

pTRE-Myc is a tetracycline response plasmid that can be used to tag a gene of interest with a c-Myc epitope and then to induce expression of the tagged protein in Tet-On™, Tet-Off™, RevTet-On™, or RevTet-Off™ Cell Lines. The Tet Expression Systems and Cell Lines give researchers ready access to the tetracycline-regulated expression systems described by Gossen & Bujard (1; Tet-Off) and Gossen *et al.* (2; Tet-On). The pTRE-Myc Vector contains an MCS downstream of the Tet-responsive P_{hCMV^*-1} promoter. cDNAs or genes inserted in the MCS will be responsive to either the tTA or rtTA regulatory protein when expressed by a separate construct in the Tet-Off or Tet-On systems, respectively. The P_{hCMV^*-1} promoter contains the Tet-responsive element (TRE), which consists of seven copies of the 42-bp tet operator sequence (*tetO*). The TRE element is just upstream of the minimal CMV promoter ($P_{min CMV}$), which lacks the enhancer that is part of the complete CMV promoter in the pTet plasmids. Consequently, P_{hCMV^*-1} is silent in the absence of binding of TetR or rTetR to the *tetO* sequences. In addition to these elements, pTRE-Myc includes a Kozak consensus ribosome binding site (3), an ATG initiation codon, and the Myc epitope directly upstream of the MCS; thus, these elements should not be included in the cloned insert. cDNAs inserted in-frame will be expressed as N-terminal Myc-tagged fusions, permitting detection using CLONTECH's c-Myc Monoclonal Antibody (#3800-1). To permit selection of stable transfectants, this plasmid should be cotransfected with the pTK-Hyg Vector (not included).

The pTRE-Myc-Luc Control Vector, packaged with the pTRE-Myc Vector, contains an additional 1,660 bp encoding firefly luciferase inserted into the *Hind III* site of the MCS. This vector can be used as a reporter of induction efficiency using standard luciferase detection reagents. It is not intended as a cloning vector. In Western blots, myc-luciferase can be detected as a 68-kDa protein.

Location of features:

- $P_{\text{hCMV}^{-1}}$ Tet-responsive promoter: 7–438
Tet-responsive element (TRE): 7–318
Location of seven *tetO* 18-mers: 15–33; 57–75; 99–117; 141–159; 183–201; 225–243 & 257–275
Fragment containing $P_{\text{min CMV}}$: 319–438
TATAA box: 341–348
- Tagging/cloning sequence 504–618
Start codon (ATG) 504–506
c-Myc epitope: 507–542
Multiple cloning site (MCS): 548–618
- Fragment containing β -globin poly A signal: 638–1787
- Fragment containing Col E1 origin of replication: 1978–2632
- Ampicillin resistance gene (β -lactamase): 3709–2779
Start codon (ATG): 3639–3637
Start codon: 2781–2779

Propagation in *E. coli*:

- Suitable host strains: DH5 α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 $\mu\text{g/ml}$) to *E. coli* hosts.
- *E. coli* replication origin: Col E1

References:

1. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci USA* **89**:5547–5551.
2. Gossen, M., *et al.* (1995) *Science* **268**:1766–1769.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.

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.

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. CLONTECH products may not be resold, modified for resale, or used to manufacture commercial products without written approval of CLONTECH.

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