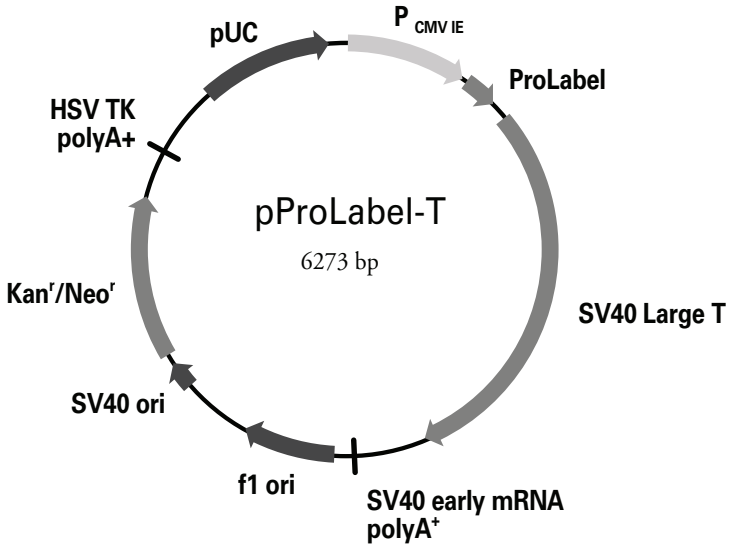
Vector Information

pProLabel-T Vector Information PT3934-5

Sold as part of Catalog No. 630458



Map of pProLabel-T Vector. All restriction sites shown are unique.

Description

pProLabel-T Vector is a mammalian expression vector encoding a fusion of the SV40 large T antigen at the C-terminus of the ProLabel tag (~6 kDa). Because ProLabel is the α fragment of the split β-galactosidase enzyme, the ProLabel-T fusion protein alone has no enzymatic activity. However, the ProLabel-T fusion can recombine with the Ω fragment of β-galactosidase to reconstitute an active enzyme. The SV40 polyadenylation signals downstream of the ProLabel-SV40 T gene direct proper processing of the 3'end of mRNA. The vector backbone also contains an SV40 origin for replication in any mammalian cell line that expresses the SV40 T antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV-TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli.* The pProLabel-C vector backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

In mammalian cells, this vector expresses the ProLabel-T fusion protein. The ProLabel-T fusion has no independent enzymatic activity, but when combined in solution with the Ω fragment of β-galactosidase, can reconstitute an active enzyme capable of hydrolyzing a chemiluminescent substrate to give an amplified signal. The recombinant ProLabel vector can be transfected into mammalian cells using any standard transfection method. ProLabel fusion expression levels can be monitored in cell lysates using the ProLabel assay. The pProLabel-T construct is provided in the Matchmaker™ Chemiluminescent Co-IP Vector Set (Cat. No. 630458) as a prey control construct. The ProLabel-T fusion will interact with AcGFP1-p53 but not with AcGFP1-Lamin. The ability of ProLabel-T to coimmunoprecipitate with AcGFP1-p53 can be measured using the ProLabel assay. Please refer to the ProLabel Screening Kits User Manual (PT3789-1) for additional information on detection of ProLabel fusions.

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Location of features

- CMV immediate early promoter
  Start: 1 End: 589
- ProLabel
  Start: 613 End: 780
  ProLabel Tag
- SV40 Large T
  Start: 868 End: 2736
  N-terminal truncation of SV40 Large T antigen (amino acids 87–709)
- f1 single-strand DNA origin
  Start: 3189 End: 3644
- SV40 early mRNA polyA signal
  Start: 3092 End: 3142
- SV40 origin of replication
  Start: 3985 End: 4120
- KAN/NEO resistance
  Start: 4169 End: 4963
- HSV TK polyA signal
  Start: 5199 End: 5217
- pUC plasmid replication origin
  Start: 5548 End: 6191

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Eglen, R.M. & Singh, R. (2002) Comb. Chem. High Throughput Screen **6**:381–387.
2. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, UK) pp. 143–190.

Note: 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.