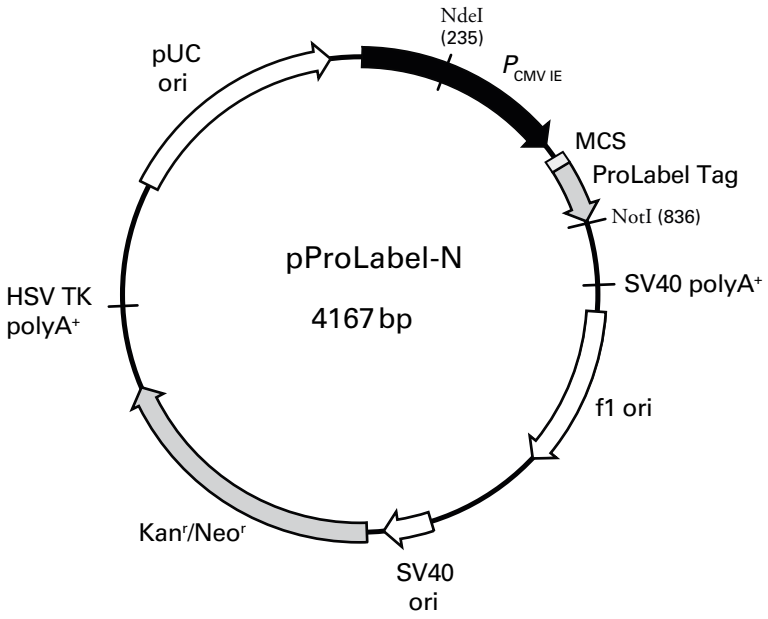
Vector Information

pProLabel-N Vector Information PT3970-5

Sold as part of Catalog No. 631628



591 **NheI** GCT AGC GCT ACC GGA CTC **BglII** AGA TCT CGA **SacI** GCT CAA **HindIII** GCT TCG AAT TCT **PstI** GCA GTC GAC
XhoI **EcoRI** **SalI**

641 **KpnI** GGT ACC **ApaI** GCG GGC CCG **BamHI** GGA TCC **Start of ProLabel** AGC TCC
SmaI

pProLabel-N Vector Map and Multiple Cloning Site (MCS). All restriction sites shown are unique.

Description

pProLabel-N is a mammalian expression vector designed to express a protein of interest fused to the the N-terminus of a 6 kDa ProLabel tag. The resulting fusion protein can be used in a variety of functional assays and quantitated with our ProLabel Detection Kit II (Cat. No. 631629). The Detection Kit provides all of the components needed to perform enzyme fragment complementation assays (1, 2). In these assays, two inactive enzyme fragments (the ProLabel tag, and a larger Enzyme Acceptor) are combined to form a complete, active enzyme that cleaves the Galacton Star chemiluminescent substrate. The resulting signal can be detected and quantitated with any standard luminometer.

The pProLabel-N vector contains a CMV promoter that drives strong, constitutive expression of the fusion protein, and an SV40 polyadenylation signal that directs processing of the 3'end of the mRNA transcript. The vector also contains a kanamycin/neomycin resistance cassette (Kan^r/Neo^r) that allows G418 selection of stably transfected eukaryotic cells; a bacterial promoter upstream of this cassette allows kanamycin or neomycin selection of transformed bacterial cells. In addition, pProLabel-N contains an SV40 origin of replication for propagation in mammalian cells that express SV40 T-antigen, a pUC origin for propagation in *E. coli* and an f1 origin for the production of single-stranded DNA.

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(021612)

Use

pProLabel-N is available in the ProLabel Quantitative Expression Vector Set (Cat. No 631628). In order to create a fusion of your protein of interest and the ProLabel tag, your gene of interest must be in the same reading frame as the ProLabel, and there can be no intervening stop codons. Prolabel vector constructs can be transfected into mammalian cells using standard transfection methods.

ProLabel fusion protein expression levels can be measured quantitatively from mammalian cell lysates using the method described in the ProLabel Detection Kit II Protocol-at-a-Glance (PT3987-2). This highly sensitive and rapid procedure obviates the need for Western analysis. The assay is designed to be used with any ProLabel fusion in a variety of functional assays—from measuring target gene expression in RNAi knockdown studies to measuring protein interactions in coimmunoprecipitation studies.

Location of features

- Human CMV immediate early promoter: 1–589
- Multiple Cloning Site (MCS): 591–665
- ProLabel Tag: 666–833
- SV40 polyA signal: 986–1036
- f1 single-strand DNA origin: 1083–1538
- SV40 origin of replication: 1879–2014
- Kan/Neo resistance: 2063–2857
- HSV TK polyA signal: 3093–3098
- pUC plasmid replication origin: 3442–4085

Propagation in *E. coli*

- Suitable host strains: DH5α, Fusion Blue, 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. Gorman, C. (1985) In *DNA cloning: A practical approach, Vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, UK) pp. 143–190.
2. Eglen, R.M. and Singh, R. (2002) Comb. Chem. High Throughput Screen. 6: 381–387.

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.