pCMV-ProLabel® C Vector

Product Code: 93-0012 **Lot Number:** See Vial

Contents: 10 µg at 0.5 µg/µl

Background

DiscoverX cell-based assays feature the powerful in vivo application of the established Enzyme Fragment Complementation (EFC) technology. In this approach, two complementing fragments; the small enzyme donor (ED) fragment and a larger enzyme acceptor (EA) fragment of the β-galactosidase (β-gal) enzyme are stably expressed as protein fusions in transfected cell lines. When ED binds to EA, complementation (EFC) occurs and β-gal activity is reconstituted and measured by substrate conversion to a luminescent product.

The pCMV-ProLabel® and pCMV-ProLink™ mammalian cloning vectors are intended for mammalian cell expression of a GPCR or other protein of interest as a fusion protein with an ED [ProLabel (PL) or ProLink (PK)] tag. The resulting ED-tagged protein, upon interacting with an EA-tagged protein (most typically co-expressed in a EA-parental cell line), gives rise to EFC. Different ProLink and ProLabel tags have different affinities for the EA protein (and in the case of ARMS-containing vectors, different affinities for Arrestin) and their selection and usage are described in detail in the pCMV-ProLabel and pCMV-ProLink Mammalian Cloning Vectors User Manual.

Product Information

Storage: Ships frozen on dry ice. Store at –20°C until use.

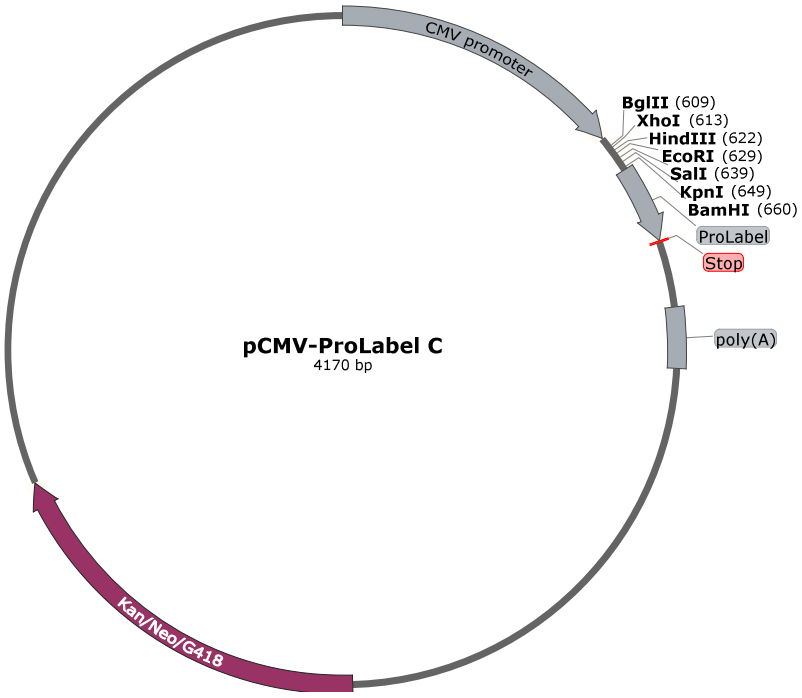
Propagation: Transform plasmid DNA into *E. coli* cells using 50 µg/ml kanamycin as the selection antibiotic.

User Manuals Pertaining To This Product:

ProLabel® and ProLink™ Mammalian Cloning Vectors (cat. no. 70-385)

PathHunter® EA Parental Cell Lines (cat. no. 70-377)

Vector Map



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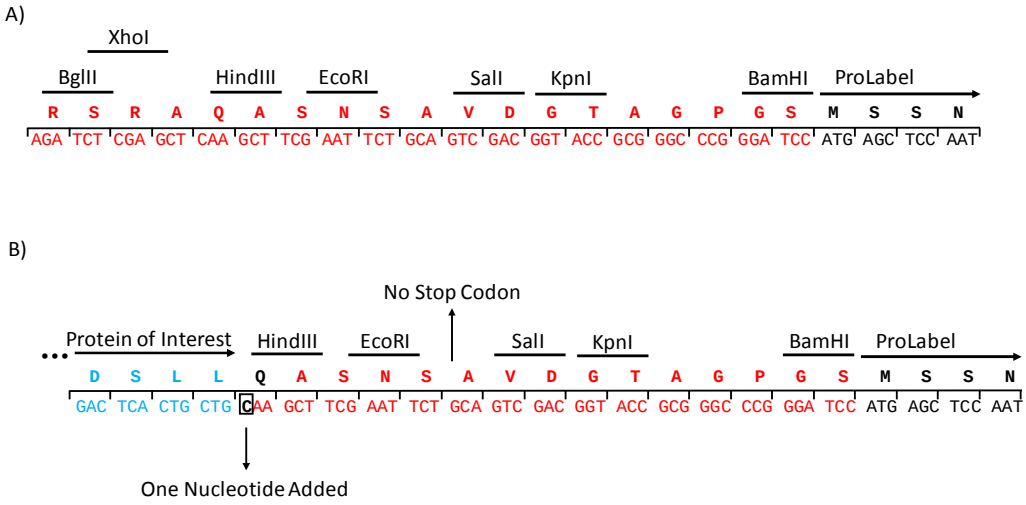
Multi-cloning Site



Successful cloning of a protein of interest fused to an ED (ProLabel® and ProLink™) tag requires that: 1) the protein of interest must be cloned into this vector in the same reading frame as the ED tag and 2) there must be no in-frame stop codons between the protein of interest and the tag.

A) Available restriction sites in the multiple cloning site (MCS) are shown along with nucleotide and translated amino acids in the reading frame of the ED tag. The protein of interest must be cloned in this same reading frame.

B) For example, when designing a PCR primer to add a HindIII site to the 3' end of a protein of interest cDNA (for cloning into the HindIII site of vector MCS), it may be necessary to add 1-2 nucleotides between the protein of interest coding sequence and nucleotides AAGCTT (the HindIII site) in order to maintain the same reading frame for the insert and tag. In the example shown, addition of 1 nucleotide (boxed) was required between the 3' end of the protein of interest cDNA and the HindIII site. Use of other restriction sites may also require additions of 1-2 nucleotides. In addition, there must be no in-frame stop codons between the protein of interest and the ED tag.

For order placement or technical support, please call 1.866.448.4864 (North America) or +44.121.260.6142 (Europe) or e-mail info@discoverx.com. For additional information, please visit www.discoverx.com.

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