DiscoverX

User Manual

ProLabel® and ProLink™ Mammalian Cloning Vectors

Vectors for Expressing ProLabel- or ProLink-Tagged Fusion Proteins in EA Cell Lines

Please read entire booklet before proceeding with the assay.
For additional information or Technical Support see contact information below.

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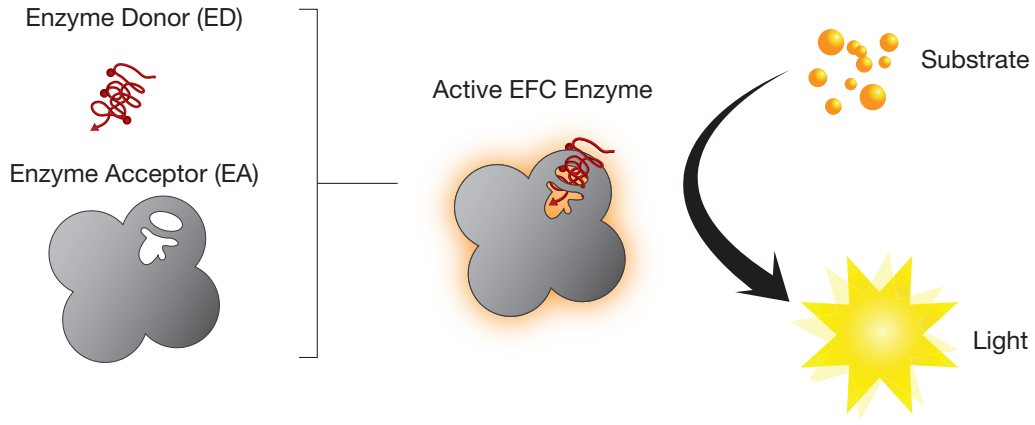
Overview

DiscoverX cell-based products feature the powerful application of the established Enzyme Fragment Complementation (EFC) technology pioneered by DiscoverX. In this approach, two complementing fragments, a small enzyme donor (ED) fragment and a larger enzyme acceptor (EA) fragment, of a β-galactosidase (β-gal) enzyme are expressed as protein fusions in stably transfected, clonally derived cells. The two fragments, ED and EA, are inactive when apart. However, when they interact, complementation occurs, and they form a functional enzyme that hydrolyzes a β-gal substrate to generate a chemiluminescent signal. In this system, ED is also referred to more specifically by its different peptide forms called enhanced ProLabel (ePL) or ProLink (PK1 and PK2).



Intended Use

pCMV Mammalian cloning vectors are intended for cloning your protein of interest (e.g. membrane protein or intracellular protein) with ePL, PK1 or PK2, as a N- or C-terminal tag, and subsequently transfecting into a parental cell line expressing an EA-tagged reporter protein (Note: in some assay formats the cells only express an ED protein and the EA protein is added to the assay at the time of detection).

ePL and PK tags have related but distinct amino acid sequences, each of which has been optimized for different applications. Refer to the table below for additional information for each cloning vector.

ePL/PK Tag Cloning Vectors Information			
Vector Name	**Vector Details**	**Protein Tagging**	**Typical Application**
pCMV-ProLabel N	High affinity enhanced PL tag	N-Terminus	Nuclear translocation
pCMV-ProLabel C	High affinity enhanced PL tag	C-Terminus	Nuclear translocation
pCMV-ProLink 1	Low affinity PK1 tag	C-Terminus	Standard protein:protein interaction
pCMV-ProLink 2	High affinity (~3-fold greater) PK2 tag	C-Terminus	Weak protein:protein interactions
pCMV-ARMS1-ProLink 2	High affinity PK2 tag; ARMS1* sequence	C-Terminus	GPCRs; Improve signal-to-background (S:B) ratios by addition of an ARMS1 sequence
pCMV-ARMS2-ProLink 2	High affinity PK2 tag; ARMS2 sequence	C-Terminus	GPCRs; Improve S:B ratios by addition of ARMS2 sequence

*ARMS (Arrestin Recruitment Modulating Sequence) is an 18-21 amino acid sequence between the GPCR and the PK tag that has been shown to enhance β-arrestin recruitment thus improving S:B in PathHunter assays. ARMS1 and ARMS2 are different variants of the 18-21 amino acid sequence.

Materials Provided and Storage Conditions

Each pCMV Mammalian cloning vector contains 10 µg of pCMV-ProLink plasmid DNA (frozen) as specified on the tube label and individual vector datasheet. Store vectors at –20°C until use.

Vectors and Vector Bundles	
Product Description	**Cat. No.**
pCMV-ProLabel N Vector	93-0009
pCMV-ProLabel C Vector	93-0012
ProLabel Cloning Vector Bundle (2-Pack) (Includes pCMV-ProLabel N Vector and pCMV-ProLabel C Vector)	93-1109
pCMV-ProLink 1 Vector	93-0167
pCMV-ProLink 2 Vector	93-0171
pCMV-ARMS1-ProLink 2 Vector	93-0489
pCMV-ARMS2-ProLink 2 Vector	93-0490
ProLink Cloning Vector Bundle (4-Pack) (Includes pCMV-ProLink 1 Vector, pCMV-ProLink 2 Vector, pCMV-ARMS1-ProLink 2 Vector, and pCMV-ARMS2-ProLink 2 Vector)	93-0491

Additional Materials Needed

To detect the interaction between ED (ePL, PK1 or PK2) and EA, you will need a specific detection kit that includes the substrate (and possibly also contains EA depending on the cell line and assay).

Please refer to cell line datasheet for the appropriate detection kit.

Cloning Information

The following cloning information are for both enhanced ProLabel (ePL) and ProLink (PK) vectors.

Propagation

Use 50 µg/mL Kanamycin for propagating plasmid DNA in *E. coli* cells.

Choice of Tag

Several variations of the ED tags provide additional options for the user and extend the system's versatility for assay development (see ePL/PK Tag Cloning Vectors Information). The binding affinity of PK1, PK2, and ePL tags for the EA fragments increase in the order written. PK1 and PK2 are used for strong and weak protein-protein interactions, respectively, while ePL (or sometimes PK2) is typically used for nuclear translocation assays. For GPCR β-arrestin2 recruitment assays, the addition of Arrestin Recruitment Modulating Sequences, ARMS1 and ARMS2, may improve the interaction with β-arrestin2 thus improving S:B in PathHunter® assays.

Placement of Tag

In considering where to place the ED tag on a membrane protein, the ED tag must have an intracellular, cytoplasm-facing orientation (to allow interactions with the nuclear, membrane, and endosomal EA-reporter proteins). For some protein classes (such as GPCRs or type I/II membrane proteins), protein topology absolutely constrains the choice of tag placement to one end or the other. However, owing to their small size, the accessibility and function of the ED tag may be affected by its location in relation to the folded target protein or membrane (e.g. amino- versus carboxyl-terminal fusion). Thus, for soluble proteins (or for membrane proteins where N- and C-termini are in- or facing— the 'cytoplasmic' compartment) it may be beneficial to empirically determine whether N- or C-terminal tagging with ED gives better assay performance. In summary, where target protein properties allow consideration of both N- and C-terminal tag fusions (e.g. soluble proteins), empirical determination of the preferred tag placement is recommended. The currently available ProLink vectors place the small ED tag at the C-terminus of the target protein, while the ProLabel vectors come in formats allowing placement of the ED tag at either the C- or the N-terminus of the target protein. To inquire about availability of additional ProLink vectors, please contact DiscoverX technical support.

Considerations for Cloning

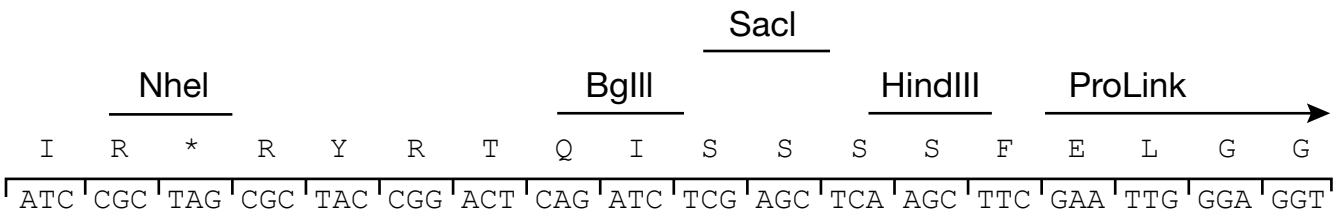
For C-terminal tag vectors: All ProLink and ProLabel C vectors do not contain a start codon, and thus a proper start codon and Kozak sequence should be present in the target protein of interest. DiscoverX uses ACC (Kozak sequence) immediately prior to the ATG start codon to enhance expression. These vectors do supply a stop codon after the C-terminal ED tag.

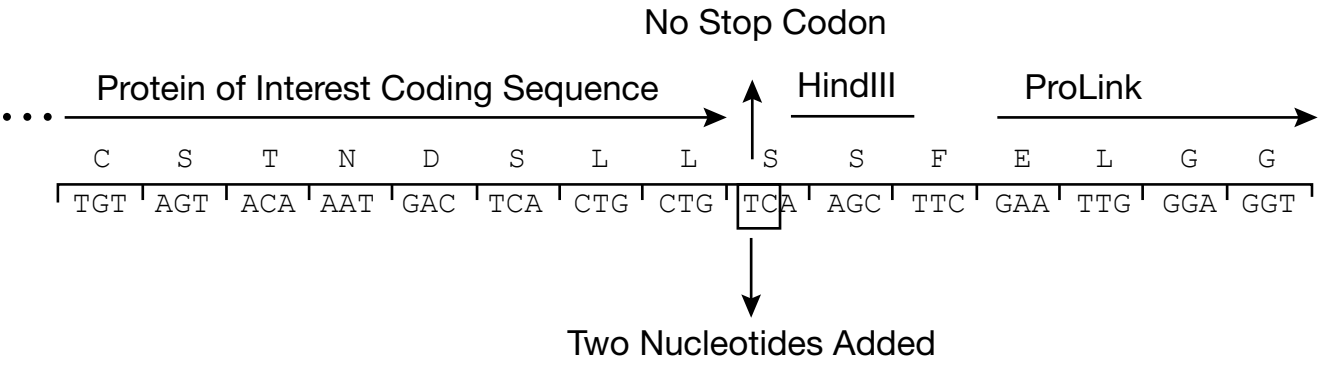
For N-terminal tag vectors: The ProLabel N vectors do contain a start codon and Kozak sequence so this is not needed in the target protein insert. Stop codons (in all 3 reading frames) are present downstream of the multiple cloning site (MSC) making a stop codon in the target protein insert non-essential.

For both N- and C- vectors: Care must be taken to ensure that the target protein insert is in the same translational reading frame as the ED tag and that no intervening stop codons are present. Cloning into available restriction sites in the pCMV-ProLink and ProLabel vectors MCSs may require nucleotide additions to maintain the open reading frame between the ED tag and the target protein. See the example on the next page for cloning into the HindIII site of a C-terminally ED tagged vector. When using an N-terminally tagged vector, the concept is identical, with the only difference being the relative positions of the tag and the target proteins are reversed. Using Lac Z as Gene-X is not recommended. All vectors use the CMV promoter for expression in a broad range of mammalian cell types.

For individual vector maps and multiple cloning site sequences, please refer to the specific vector datasheet.

The two figures below show examples of (top image) a typical MSC and (lower image) insertion of a GPCR/protein of interest into the HindIII site in the same reading frame as the ED tag. Additionally, the lower image shows how nucleotides may need to be added, and stop codons excluded, between the GPCR/protein of interest and the ED tag to maintain the open reading frame. Before engineering your construct, it is essential to refer to the specific vector datasheet for details of the actual multiple cloning site and other vector details.





C-terminal tags: When designing a PCR primer for the 3' end of the GPCR (or protein of interest), the GPCR stop codon must be removed. Primers must also be designed for in-frame fusion with PK tag. For example, when using the Hind III site, add two nucleotides before the AAGCTT to ensure that the reading frame is maintained between the GPCR and PK. Other restriction sites require nucleotide additions as well to maintain reading frame integrity.

N-terminal tags: PCR primers for the 5' end of the protein of interest, must be designed to yield in-frame fusion with upstream PK tag. Analogous with the example above, nucleotide additions may be required to maintain reading frame integrity.

For additional information or technical support, please contact Technical Support listed below.

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