pACP-tag(m)-2 Vector

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N9322S 001101012100

N9322S

20 µg	Lot: 0011010
Store at: –20°C	Exp: 10/12

Introduction

pACP-tag(m)-2 Vector is a mammalian expression plasmid encoding ACPwt, an ACP-tag protein, which is expressed under control of the CMV promoter. The expression plasmid has an IRES (Internal Ribosome Entry Site) and a neomycin resistance gene downstream of the ACP-tag for the efficient selection of stable transfectants. This plasmid is intended for the cloning and transient or stable expression of ACP-tag protein fusions in mammalian cells. pACP-tag(m)-2 contains two multiple cloning sites to allow cloning of the fusion partner as a fusion to the C-terminus of the ACP-tag and an appropriate signal peptide to the N-terminus of the ACP-tag.

The ACP-tag is a small protein tag (8 kDa) based on the acyl carrier protein (ACP). It allows the specific, covalent attachment of virtually any molecule to a protein of interest. ACP-tag substrates are derivatives of coenzyme A (CoA). In the labeling reaction, the substituted phosphopantetheine group of CoA is covalently attached to a conserved serine residue of the ACP-tag by a phosphopantetheine transferase (ACP or SFP Synthase). Having no cysteines, the ACP-tag is particularly suited for specifically labeling cell-surface proteins, and should be useful for labeling secreted proteins with disulfide bridges such as antibodies.

There are two steps to using this system: subcloning and expression of the protein of interest as an ACP-tag fusion, and labeling of the fusion protein with the CoA substrate of choice. In this document, the cloning and expression of ACP-tag protein fusions is described. The labeling of fusion proteins with CoA substrates is described in the documentation supplied with CoA substrates and ACP or SFP Synthase.

Materials Required but not Supplied:

Mammalian cell lines

Transfection reagents

CoA substrates

ACP or SFP Synthase

Tissue culture reagents and media

Storage

pACP-tag(m)-2 is supplied in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) at a concentration of 0.5 µg/µl. Plasmid solutions can be stored at 4°C for up to one week. For long-term storage –20°C is recommended.

Detailed Description

A plasmid map and the sequence of the cloning region can be found at the end of these instructions. The complete plasmid sequence can be downloaded at www.neb.com. This plasmid encodes the gene ACPwt which is the wild type Acyl Carrier Protein from E. coli. In the plasmid sequence, the ACPwt gene is encoded from bp 975 to 1208. The ACPwt gene was cloned via EcoRI and SbfI including additional flanking restriction sites.

This plasmid is intended for the cloning and stable or transient expression of ACP-tag protein fusions in mammalian cells. It is suitable for the efficient production of stable cell lines expressing ACP-tag gene fusions. The plasmid contains the CMV promoter followed by the genes for the ACP-tag and neomycin resistance separated by the IRES of the encephalomyocarditis virus (ECMV), which permits the translation of two open reading frames from one messenger RNA; therefore after selection of stable mammalian cells for neomycin resistance, nearly all surviving colonies should stably express the ACP-tag fusion protein. Unless the expression experiments require a pure population of cells, the pool of resistant cells can simply be used, otherwise cell clones can be isolated and characterized using standard procedures.

The plasmid contains the β-lactamase (Ampicillin resistance) gene for maintenance in bacteria. The gene of interest should be cloned downstream of the ACP-tag coding sequence, as a fusion to the C-terminus of the ACP-tag. An appropriate cell surface signal peptide should be cloned upstream of the ACP-tag as an N-terminal fusion. A Kozak sequence is located upstream of the ACPwt gene to increase the translation efficiency of the fusion protein. The ACPwt gene can be isolated from the plasmid using PCR or direct cloning in order to subclone it into a different vector of choice.

Cloning of ACP-tag Fusions in pACP-tag(m)-2

Cloning by PCR

To subclone a gene of interest or an appropriate cell surface signal peptide into pACP-tag(m)-2 to create an N-terminal fusion to the ACP-tag, use the available restriction sites: NheI, EcoRV(blunt), AscI, SwaI (blunt), BsrGI, AgeI or EcoRI which are located upstream of the ACP-tag.

To subclone the gene of interest into pACP-tag(m)-2 to create a C-terminal fusion to the ACP-tag, use the available restriction sites downstream of the ACP-tag: SbfI, BamHI, PmeI(blunt), XhoI, PacI and NotI.

Notes:
When making a C-terminal fusion to the ACP-tag, note that there is a stop codon between the PacI and NotI sites, so SbfI, BamHI, PmeI, XhoI or PacI must be used as the 5´ cloning site for the insert.

PmeI and XhoI cannot be used together for cloning because they share a cytosine as part of their recognition sequences.

Primer Design and Cloning Considerations:

- Design the PCR primers to include a sufficient overlap (15–20 bp) with the sequence of the gene to be amplified.
- For fusion to the C-terminus of the ACP-tag, a stop codon may be included at the C-terminus of the fusion (in front of the downstream cloning site) in order to terminate translation at that position.
- For fusions upstream of the ACP-tag, ensure that a start codon is included. The addition of a Kozak sequence (e.g. GCCRCCATG, where the start codon is underlined) will increase the translation efficiency.
- In general, any linker peptide between the proteins should be kept short to avoid degradation by proteases. If required, specific protease cleavage sites can be introduced into the linker peptide.
- Care should be taken to design the cloning so that the fusion partners in the resulting construct are in frame.
- Perform the PCR reaction and subsequent cloning steps according to established protocols for molecular biology.
- After subcloning the gene of interest into pACP-tag(m)-2 as a fusion with the ACPwt gene, the resulting plasmid can be used for stable or transient expression of the ACP-tag fusion proteins in a suitable cell line.

Direct Cloning

Direct cloning can also be used to make fusions with the ACP-tag. This is only possible if the fusion partner has compatible sites adjacent to the gene of interest.

Care should be taken to design the cloning so that the fusion partners in the resulting construct are in frame.

Note: When making a C-terminal fusion to the ACP-tag, note that there is a stop codon between the PacI and NotI sites, so SbfI, BamHI, PmeI, XhoI or PacI must be used as the 5´ cloning site for the insert.

PmeI and XhoI cannot be used together for cloning because they share a cytosine as part of their recognition sequences.

Expression of ACP-tag Fusions

Transient Expression

Expression of the fusion protein cloned in pACP-tag(m)-2 can be achieved by transiently transfecting cells in culture with standard transfection protocols. The appropriate reagent and time to permit adequate expression must be empirically determined. ACP-tag fusion proteins can be observed 24 hours post-transfection. We recommend using pACP-ADRβ2 as an expression control plasmid.

ACP-ADRβ2 fusion protein gives a cell surface localized signal when labeled with CoA substrates.

pACP-ADRβ2 has performed well in stable and transient transfection of CHO-K1, COS-7, U-2 OS and NIH-3T3 cells. Note that the intensity of the fluorescence may vary, depending on the cell line and labeling substrate used.

Stable Expression

pACP-tag(m)-2 can be transfected by standard transfection methods. Twenty-four to 48 hours after transfection, begin selecting mammalian cultures in 600–1,200 µg/ml G418 (geneticin) depending on the cell line. It is recommended that a kill curve be established for each cell line to determine optimal selection conditions. After 8–12 days of continuous selection, stable colonies will become visible. It is possible to use pools of stable cell populations for initial cell labeling to test for the presence of ACP-tag expression. In addition, monoclonal cell lines can be isolated and characterized, if desired.

(see other side)

Troubleshooting

Cloning of the Gene of Interest

If subcloning of the gene of interest with the ACP-tag does not work, reconfirm all the cloning steps (primer design, choice of restriction site, etc.). If all steps are confirmed as being correct, then try the cloning using different restriction sites. Be sure to include a positive and negative control for the ligation reaction.

Alternatively, try to subclone the ACP-tag gene into an expression vector already containing the gene of interest.

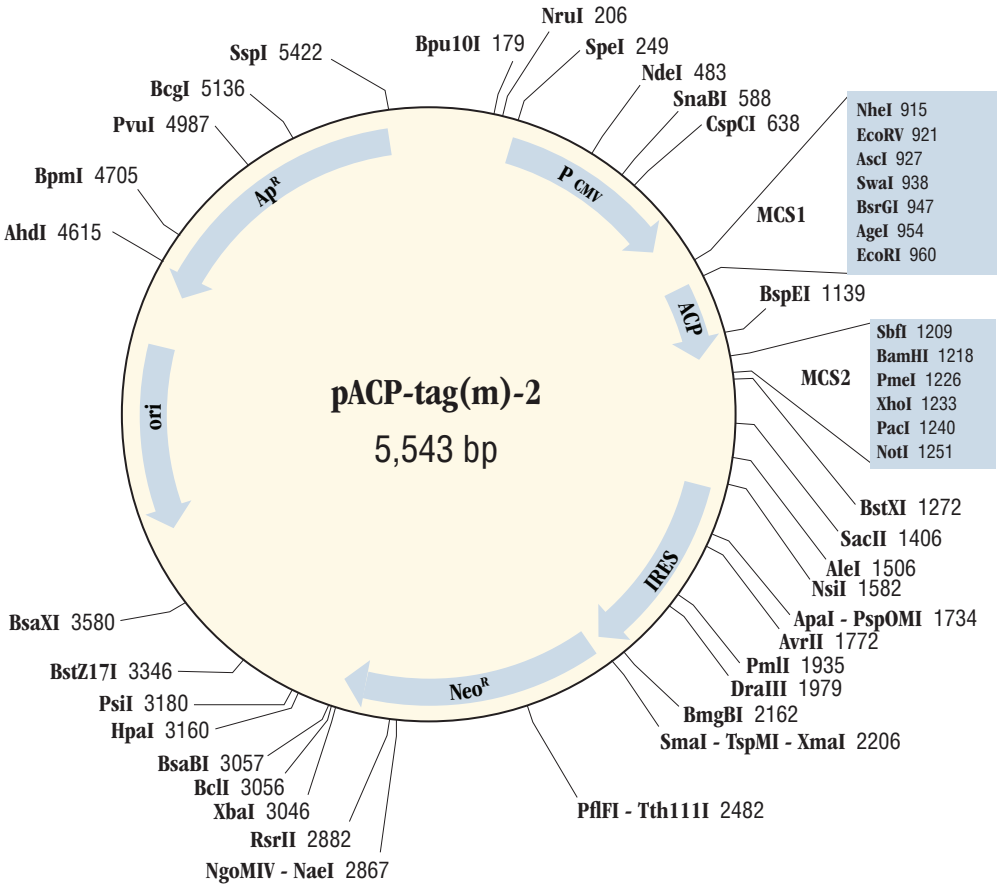
Expression

In general, we have not experienced problems expressing ACP-tag protein fusions. However, if the fusion gene does not appear to be expressed, try expressing the ACP-ADRβ2 protein fusion as a positive control using cells transiently transfected with the pACP-ADRβ2 Control Plasmid (NEB #N9321). Labeling of such cells with a fluorescent CoA substrate should show strong membrane fluorescence. Note that the intensity of this fluorescence may vary depending on cell-line and substrate used.

If the pACP-ADRβ2 construct is expressed but the fusion protein is not, then there are a variety of possible causes. It is possible that this fusion protein may be toxic for the cell line. It is difficult to troubleshoot such instances, but the use of a different expression plasmid or cell line may help. Signs of host cell toxicity could include slow proliferation or apoptosis. Counterstaining live cells with Hoechst 33342 or fixed cells with DAPI can be used to determine whether nuclei are healthy if toxicity is suspected.

Plasmid Map of pACP-tag(m)-2

This map and the map for the control plasmid can be downloaded at www.neb.com



Cloning Region of pACP-tag(m)-2

Unique restriction sites in the regions flanking the ACP-tag gene are displayed above the coding strand. The complete sequence for pACP-tag(m)-2 and pACP-ADRβ2 can be downloaded at www.neb.com

```
 721  aaaatcaacgggactttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcg

 781  gtaggcgtgtacggtgggaggtctatataagcagagctctctggctaactagagaaccca

 841  ctgcttactggcttatcgaaattaatacgactcactatagggagacccaagcttggtacc
                                             MCS1
                           ------------------------------------------------>
                            NheI   EcoRV   AscI         SwaI    BsrGI   AgeI
 901  gagctcggatcgtcGCTAGCGATATCGGCGCGCCAGCATTTAAATCTGTACAGACCGGTG
                     A  S  D  I  G  A  P  A  F  K  S  V  Q  T  G
      ------>
       EcoRI
 961  AATTCaagcttaccatgagcactatcgaagaacgcgttaagaaaattatcggcgaacagc
      E  F  K  L  T  M  S  T  I  E  E  R  V  K  K  I  I  G  E  Q

1021  tgggcgttaagcaggaagaagttaccaacaatgcttctttcgttgaagacctgggcgcgg
      L  G  V  K  Q  E  E  V  T  N  N  A  S  F  V  E  D  L  G  A

1081  attctcttgacaccgttgagctggtaatggctctggaagaagagtttgatactgagattc
      D  S  L  D  T  V  E  L  V  M  A  L  E  E  E  F  D  T  E  I

1141  cggacgaagaagctgagaaaatcaccaccgttcaggctgccattgattacatcaacggcc
      P  D  E  E  A  E  K  I  T  T  V  Q  A  A  I  D  Y  I  N  G
                                       MCS2
                ------------------------------------------------->
                 SbfI    BamHI   PmeI         XhoI    PacI     NotI
1201  accaggcgCCTGCAGGCGGATCCGCGTTTAAACTCGAGGTTAATTAATGAGCGGCCGCata
      H  Q  A  P  A  G  G  S  A  F  K  L  E  V  N  *

1261  gataactgatccagtgtgctggaattaattcgctgtctgcgagggccagctgttggggtga

1321  gtactccctctcaaaagcgggcatgacttctgcgctaagattgtcagtttcc
```

5´ MCS

```
     NheI EcoRV    AscI          SwaI     BsrGI    AgeI  EcoRI
GTCGCTAGCGATATCGGCGCGCCAGCATTTAAATCTGTACAGACCGGTGAATTCACC
CAGCGATCGCTATAGCCGCGCGGTCGTAAATTTAGACATGTCTGGCCACTTAAGTGG
R  R     R  Y  R  R  A  S  I     I  C  T  D  R     I  H
 V  A  S  D  I  G  A  P  A  F  K  S  V  Q  T  G  E  F  T
  S  L  A  I  S  A  R  Q  H  L  N  L  Y  R  P  V  N  S  P
```

3´ MCS

```
     SbfI BamHI      PmeI XhoI      PacI     NotI
CCTGCAGGCGGATCCGCGTTTAAACTCGAGGTTAATTAATGAGCGGCCGC
GGACGTCCGCCTAGGCGCAAATTTGAGCTCCAATTAATTACTCGCCGGCG
 P  A  G  G  S  A  F  K  L  E  V  N        A  A  A
  L  Q  A  D  P  R  L  N  S  R  L  I  N  E  R  P
P  C  R  R  I  R  V     T  R  G     L  M  S  G  R
```

Companion Products:

pACP-ADRβ2 Control Plasmid
#N9321S 20 µg

ACP Synthase
#P9301S 25 nmol

SFP Synthase
#P9302S 25 nmol

pMCP-tag(m) Vector
#N9317S 20 µg

pMCP-GPI Control Plasmid
#N9320S 20 µg

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices