

pMCP-tag(m) Vector



N9317S 001120615061

N9317S

20 µg

Lot: 0011206

Store at: -20°C

Exp: 6/15

Introduction

pMCP-tag(m) Vector is a mammalian expression plasmid encoding MCP, an MCP-tag polypeptide, which is expressed under control of the CMV promoter. This plasmid is intended for the cloning and transient expression of MCP-tag protein fusions in mammalian cells. pMCP-tag(m) Vector contains two multiple cloning sites to allow cloning of the fusion partner as a fusion to the C-Terminus of the MCP-tag and an appropriate signal peptide to the N-Terminus of the MCP-tag.

The MCP-tag is a polypeptide tag (8 kDa) based on the acyl carrier protein (ACP) containing two mutations (D36-T36 and D39-G39), for labeling cell membrane proteins. It allows the specific, covalent attachment of virtually any molecule to a protein of interest. MCP-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 MCP-tag by SFP-Synthase. Having no cysteines, the MCP-tag is particularly suited for specifically labeling cell-surface proteins, and should be useful for labeling secreted proteins with disulphide bridges such as antibodies.

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

Materials Required but not Supplied:

Mammalian cell lines
Transfection reagents
MCP-tag substrates

Storage

pMCP-tag(m) Vector 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 MCP which is a mutant carrier protein. In the plasmid sequence, the MCP gene is encoded from bp 690 to 923. The MCP gene was cloned via HindIII and PstI including additional flanking restriction sites.

This plasmid is intended as a transient expression vector for MCP-tag gene fusions in mammalian cell lines. It is not recommended for the production of stable cell lines. The plasmid contains β-lactamase (Ampicillin resistance) gene for maintenance in bacteria. The plasmid also contains a CMV promoter and SV40 polyadenylation signals for correct mRNA processing. The gene of interest should be cloned downstream of the MCP-tag coding sequence, as a fusion to the C-Terminus of the MCP-tag. An appropriate cell surface signal peptide should be cloned upstream of the MCP-tag as an N-Terminal fusion. A Kozak sequence is located upstream of the MCP gene to increase the translation efficiency of the fusion protein. The MCP gene can be isolated from the plasmid using PCR or direct cloning in order to subclone it into a different vector system of choice.

Cloning of MCP-tag Fusions in pMCP-tag(m) Vector

Cloning by PCR

To subclone an appropriate cell surface signal peptide into pMCP-tag(m) Vector creating an N-Terminal fusion to the MCP-tag, use the available restriction sites SacI, SacII, NotI, EcoRV or HindIII which are located upstream of the MCP-tag.

To subclone the gene of interest into pMCP-tag(m) Vector creating a C-Terminal fusion to the MCP-tag, use the available restriction sites downstream of the MCP-tag: SbfI (PstI), AscI, BamHI, XhoI, ApaI and KpnI.

Note: When making a C-Terminal fusion to the MCP-tag, note that there is a stop codon between the BamHI and XhoI sites, so SbfI (PstI), AscI, or BamHI must be used as the 5' cloning site for your insert.

Primer Design and Cloning Hints:

- Design your PCR primers to include a sufficient overlap with the sequence of the gene you want to amplify.
- You may also want to include a stop codon 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 MCP-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 pMCP-tag(m) Vector as a fusion with the MCP gene, the resulting plasmid can be used for transient expression of the MCP-tag fusion proteins in a suitable cell line.

Direct Cloning

Direct cloning can also be used to make fusions with the MCP-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 MCP-tag, note that there is a stop codon between the BamHI and XhoI sites, so SbfI (PstI), AscI, or BamHI must be used as the 5' cloning site for your insert.

Expression of MCP Fusions

Transient expression

Expression of the fusion protein cloned in pMCP-tag(m) Vector 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.

Troubleshooting

Cloning of the Gene of Interest

If subcloning of your gene of interest with the MCP-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 control for the ligation reaction.

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

Expression

In general we have not experienced problems expressing MCP-tag protein fusions. However if your fusion gene does not appear to be expressed, try expressing the pMCP-GPI Control Plasmid (NEB #N9320S). Labeling of such cells with a fluorescent CoA Substrate should show membrane fluorescence. Note that the intensity of this fluorescence may vary depending on cell line and substrate used.

If the MCP-GPI construct is expressed but your fusion protein is not, then there are a variety of possible causes. It is possible that this fusion protein may be toxic for your 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.

(see other side)

CERTIFICATE OF ANALYSIS

