

# pMCP-GPI Control Plasmid



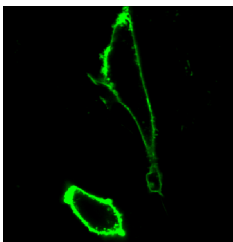
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N9320S 001120915091

## N9320S

**20 µg**                      **Lot: 0011209**  
**Store at: -20°C**        **Exp: 9/15**



*Live CHO-K1 cells transiently transfected with pMCP-GPI. Cells were labeled with CoA 488 (green) using SFP Synthase for 30 minutes.*

### Introduction

This control plasmid contains a glycosylphosphatidylinositol (GPI) anchor sequence cloned upstream of the MCP-tag coding sequence in pMCP. This sequence directs the fusion protein to the plasma membrane with the MCP tag exposed on the outside surface, giving a localized control signal when labeled with CoA substrates. GPI is present in all eukaryotic cells where it anchors glycoproteins to the cell-surface. The GPI anchor helps increase the mobility of membrane proteins thereby supporting signal transduction and cellular transport. In addition, the GPI anchor plays an important role in creating antigens on cell surfaces. The full sequence and map for pMCP-GPI can be downloaded at [www.neb.com](http://www.neb.com).

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 a phosphopantetheine

transferase (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 an 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 documents provided with the pMCP cloning plasmid. The labeling of fusion proteins with MCP-tag substrates is described in the documentation supplied with MCP-tag substrates and SFP Synthase.

### Materials Required but not Supplied:

- Mammalian cell lines
- Transfection reagents
- MCP-tag substrates
- SFP Synthase

### Storage

pMCP-GPI 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.

### Expression of MCP-GPI

#### Transient expression

Expression of MCP-GPI 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. pMCP-GPI has performed well in transient transfection of CHO-K1, Cos-7, U-205 and NIH 3T3 cells. In most cases, MCP-GPI can be observed within 24 hours post-transfection. This plasmid is not suitable for stable transfection.

### Troubleshooting

#### Expression

In general we have not experienced problems expressing MCP-GPI from the pACP-GPI plasmid. Labeling of transfected cells with a fluorescent CoA substrate should show strong surface fluorescence. Note that the intensity of this fluorescence may vary depending on cell line and substrate used.

### Note:

NEB 10-beta Competent *E. coli* (High Efficiency) (NEB #C3019) is recommended for propagating this control plasmid.

The CMV promoter is covered under U.S. Patent No. 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

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