pACP-ADRβ2 Control Plasmid

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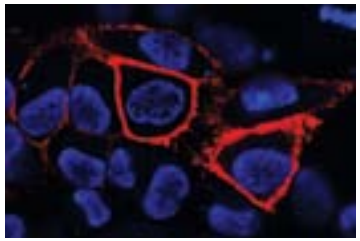
N9321S 001101012100

N9321S

20 µg **Lot: 0011010**

Store at: –20°C **Exp: 10/12**



Live U-2 0S cells transiently transfected with pACP-ADRβ2. Cells were labeled with CoA 547 using SFP Synthase for 30 minutes and imaged by confocal microscopy.

Introduction

This control plasmid contains the gene encoding the Beta-2 Adrenergic Receptor cloned as a fusion to the C-terminus of the ACP-tag. The signal peptide fused to the N-terminus of ACP-tag is based on the 5HT3A Serotonin Receptor. The Beta-2 Adrenergic Receptor is a member of the G protein coupled receptors and mediates the catecholamine-induced activation of adenylate cyclase through the action of G proteins.

The ACP-Beta-2 Adrenergic Receptor is inserted in the plasma membrane with the ACP-tag exposed to the extracellular side of the membrane. When labeled with CoA substrates, it gives a selective cell membrane fluorescence labeling pattern.The full sequence and map for pACP-ADRβ2 can be downloaded at www.neb.com.

The ACP-tag is a polypeptide 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. The cloning and expression of ACP-tag protein fusions is described in documents provided with the pACP-tag(m)-2 cloning plasmid. 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

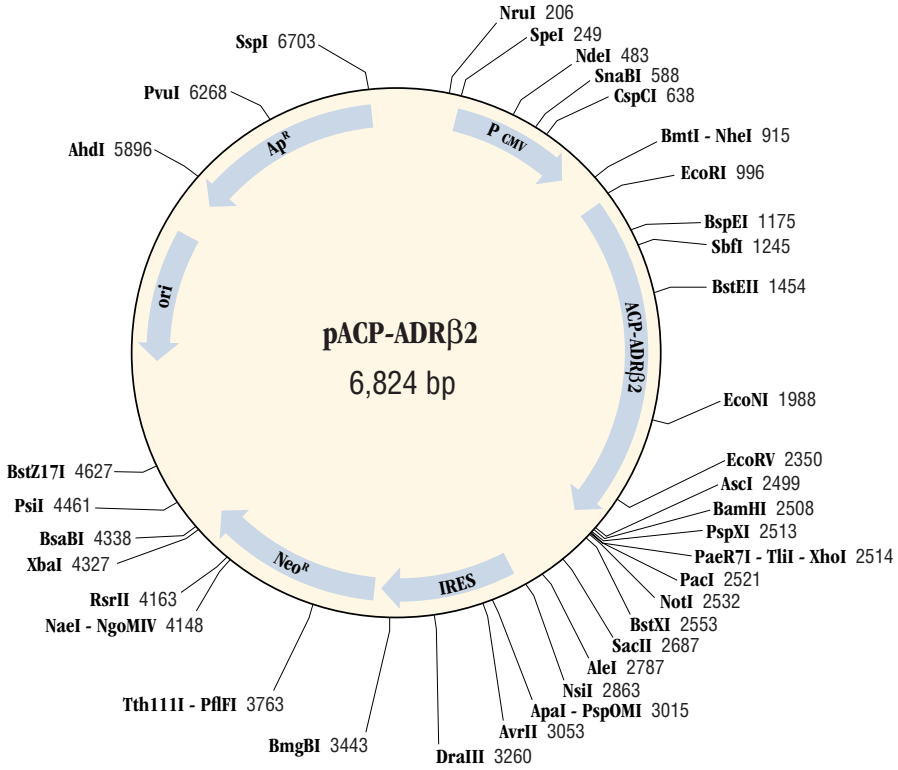
pACP-ADRβ2 is supplied in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) at a concentration of 0.5 µg/µl. Plasmid solutions can be stored at 4°C for up to one week. For long-term storage –20°C is recommended.

Expression of ACP-ADRβ2 Fusions

Transient Expression

Expression of ACP-ADRβ2 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. pACP-ADRβ2 has performed well in transient transfection of CHO-K1, COS-7, U-2 0S and NIH 3T3 cells. In most cases, ACP-ADRβ2 can be observed within 24 hours post-transfection. ACP-ADRβ2 fusion protein gives a cell surface localized signal when labeled with CoA substrates. Note that the intensity of the fluorescence may vary, depending on the cell line and labeling substrate used. This plasmid is suitable for stable transfection.

We recommend using TransPass D2 (NEB #M2554) in combination with TransPass V (NEB #M2561) or Roche's FuGENE® 6 Transfection Reagent for both transient and stable transfections.



Stable Expression

pACP-ADRβ2 can be transfected as described above for transient transfection or by other 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, clonal cell lines can be isolated and characterized if desired.

Troubleshooting

Expression

In general, we have not experienced problems expressing ACP-ADRβ2 from the pACP-ADRβ2 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. In most instances, difficulties in expression can be resolved by altering the transfection protocol.

Companion Products:

pACP-tag(m)-2 Vector
#N9322S 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

CERTIFICATE OF ANALYSIS