

pSNAP_f-ADRB₂ Control Plasmid



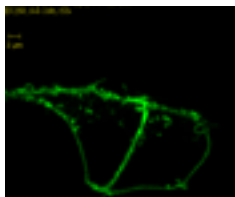
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N9184S 002111013101

N9184S

20 µg Lot: 0021110
Store at: -20°C Exp: 10/13



Live HEK293 cells transiently transfected with pSNAP_f-ADRB₂. Cells were labeled with SNAP-Surface™ 488 (green) for 15 minutes.

Introduction

This control plasmid contains the gene encoding the Beta-2 adrenergic receptor cloned downstream of the SNAP_f coding sequence in pSNAP_f as a fusion to the C-terminus of the SNAP-tag. The signal peptide fused to the N-terminus of SNAP_f 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 SNAP_f-Beta-2 adrenergic receptor is inserted in the plasma membrane with the SNAP_f exposed to the extracellular side of the membrane. When labeled with SNAP-tag substrates, it gives a selective cell membrane fluorescent labeling pattern.

The SNAP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag is a small polypeptide based on human O⁶-alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag substrates are derivatives of benzyl purines and benzyl pyrimidines. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag.

pSNAP_f contains an improved version of SNAP-tag, termed SNAP_f. SNAP_f displays faster kinetics in *in vitro* labeling and fast, specific and efficient labeling in live and fixed cell applications, thereby rendering it a desired research tool for analysis of protein dynamics.

There are two steps to using this system: sub-cloning and expression of the protein of interest as a SNAP_f fusion, and labeling of the fusion with the SNAP-tag substrate of choice. Expression of SNAP_f-ADRB₂ fusion protein is described in this document. The labeling of the fusion proteins with SNAP-tag substrates is described in the instructions supplied with SNAP-tag substrates.

Materials Required but not Supplied:

Cell culture media and reagents
Mammalian cell lines
Transfection reagents
SNAP-tag substrates

Storage

pSNAP_f-ADRB₂ 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 SNAP_f Fusions

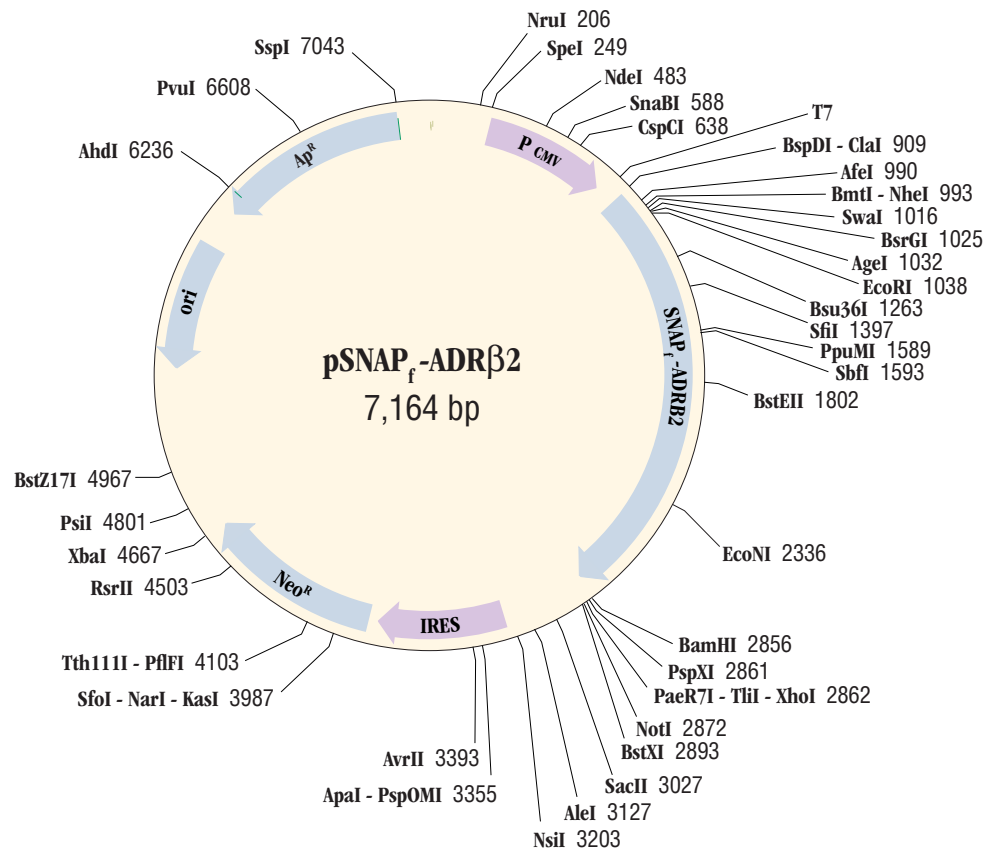
Transient Expression

Expression of the fusion protein cloned in pSNAP_f-ADRB₂ 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. pSNAP_f-ADRB₂ 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 cell line and labeling substrate used.

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

pSNAP_f-ADRB₂ 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 you establish a kill curve 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 SNAP-tag expression. In addition clonal cell lines can be isolated and characterized if desired.



Troubleshooting

Expression

In general, we have not experienced problems expressing SNAP_f-ADRB₂ from the pSNAP_f-ADRB₂ plasmid. Labeling of transfected cells with a fluorescent SNAP-Cell™ or SNAP-Surface™ substrate should show strong cell surface fluorescence. In most instances, difficulties in expression can be resolved by altering the transfection protocol.

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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