pSNAP$_f$-ADRβ2 Control Plasmid

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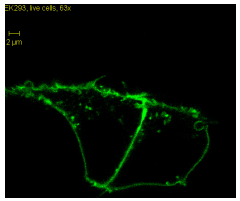
N9184S 002141117111

N9184S

20 µg **Lot: 0021411**

Store at: –20°C **Exp: 11/17**



Live HEK293 cells transiently transfected with pSNAP$_f$-ADRβ2. 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 protein based on human O^6-alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag substrates are derivatives of benzylguanines and benzylchloropyrimidines. 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$_f$-ADRβ2 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$_f$-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.

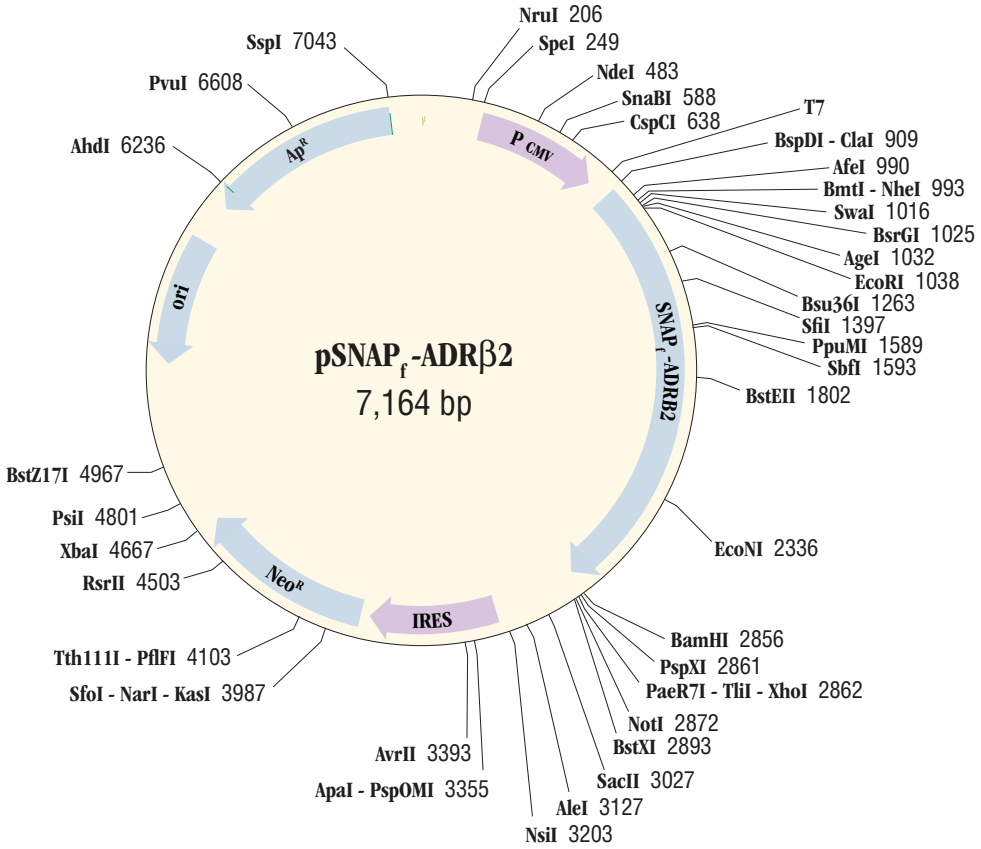
Expression of SNAP$_f$ Fusions

Transient Expression

Expression of the fusion protein cloned in pSNAP$_f$-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. pSNAP$_f$-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 cell line and labeling substrate used.

Stable Expression

pSNAP$_f$-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 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$_f$-ADRβ2 from the pSNAP$_f$-ADRβ2 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.

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The products and/or their use may be covered by one or more of the following patents and patent applications: U.S. Patent No. 7,939,284 (Methods for Using O6-Alkylguanine-DNA-Alkyltransferases); U.S. Patent No. 7,888,090 (Mutants of O6-Alkylguanine-DNA-Alkyltransferases); U.S Patent No. 8,163,479 (Specific Substrates for O6-Alkylguanine-DNA-Alkyltransferases); U.S. Patent No. 8,178,314 (Pyrimidines reacting with O6-Alkylguanine-DNA-Alkyltransferases); PCT/EP2007/057597 (Labeling of Fusion Proteins with Synthetic Probes); EP07117800 (Drug Delivery); EP07117802 (Drug Delivery); EPO7120288 (GTPase-Transient Protein Protein Interactions) These patents and patent applications are owned by Covalys, or owned by the Ecole Polytechnique Fédérale de Lausanne (EPFL) and exclusively licensed to Covalys and NEB.

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CERTIFICATE OF ANALYSIS