pSNAP$_f$-H2B Control Plasmid

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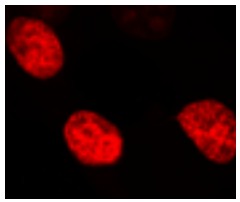
N9186S 002120615061

N9186S

20 µg **Lot: 0021206**

Store at: –20°C **Exp: 6/15**



Live COS-7 cells transiently transfected with pSNAP$_f$-H2B. Cells were labeled with SNAP-Cell® TMR-Star (red) for 15 minutes.

Introduction

This control plasmid contains the gene encoding the Histone H2B protein cloned upstream of the SNAP$_f$ coding sequence in pSNAP$_f$, as a fusion to the N- terminus of the SNAP-tag. Histone H2B is a member of the core histones that package DNA in the nucleus. The H2B-SNAP$_f$ fusion protein gives nuclear fluorescence when labeled with SNAP-Cell® substrates. The full sequence and map for pSNAP$_f$-H2B can be downloaded at www.neb.com

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 O^6-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: subcloning 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 the SNAP$_f$ -H2B fusion protein is described in this document. The labeling of 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$_f$-H2B 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

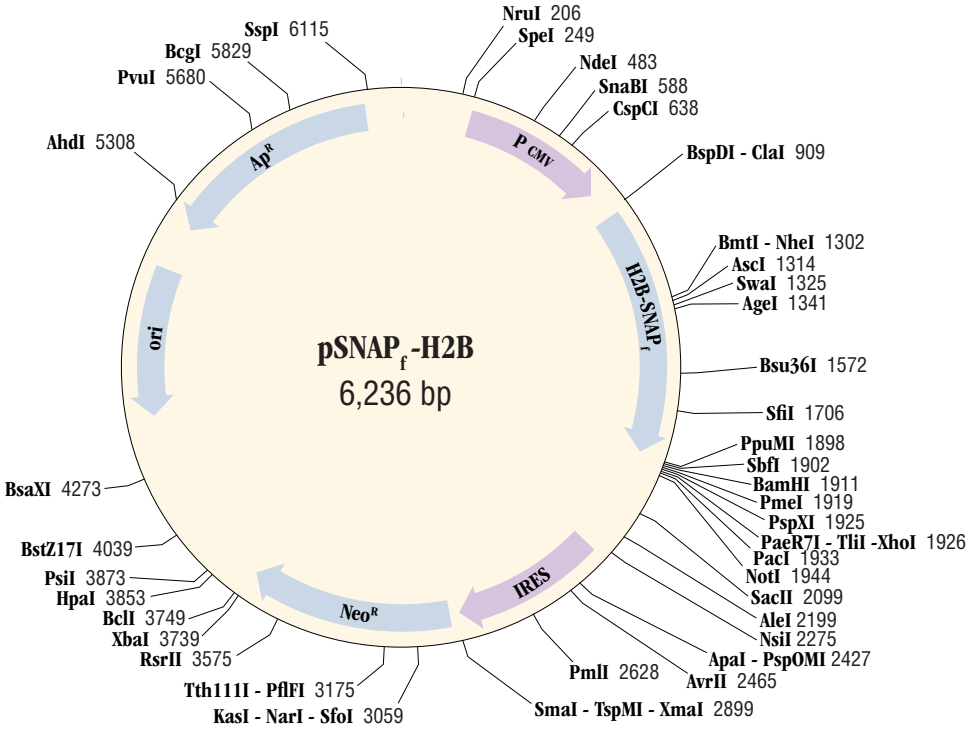
Transient Expression

Expression of the fusion protein cloned in pSNAP$_f$-H2B 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$_f$-H2B 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$_f$-H2B 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 H2B-SNAP$_f$ from the pSNAP$_f$-H2B plasmid. Labeling of transfected cells with a fluorescent SNAP-Cell substrate should show strong nuclear fluorescence. In most instances, difficulties in expression can be resolved by altering the transfection protocol.