

pSNAP_f-H2B Control Plasmid



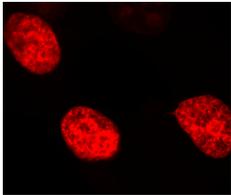
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N9186S 001120415041

N9186S

20 µg Lot: 0011204
Store at: -20°C Exp: 4/15



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 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 protein based on human O⁶-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 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.

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.

