The SNAP-tag is a small protein based on virtually any molecule to a protein of interest, allowing the specific, covalent attachment of SNAP-tag substrates.

The SNAP-tag is a novel tool for protein research, permitting adequate expression must be empirically determined. pSNAPf-H2B has performed well 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.

Materials Required but not Supplied:
- Cell culture media and reagents
- Transfection reagents
- SNAP-tag substrates

Storage
pSNAPf-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 Fusions

Transient Expression
Expression of the fusion protein cloned in pSNAPf-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. pSNAPf-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
pSNAPf-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 for the SNAP-tag substrate of choice. Expression of the SNAPf-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.

Expression
In general, we have not experienced problems expressing H2B-SNAP, from the pSNAP-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.

Troubleshooting

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