There are two steps to using this system: sub-cloning and expression of the protein of interest as a SNAP, fusion, and labeling of the fusion with the SNAP-tag substrate of choice. Expression of the SNAP, 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, 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 pSNAP, 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, 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, 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, cloning cell lines can be isolated and characterized if desired.

Troubleshooting
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.

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