Expression of SNAP Fusions (N9177)

Overview

Protocol

1. **Transient Expression**

   Expression of the fusion protein cloned in pSNAP-Cox8A 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-Cox8A 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.

2. **Stable Expression**

   pSNAP-Cox8A 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.

3. **Troubleshooting**

   Expression

   In general we have not experienced problems expressing SNAP-Cox8A from the pSNAP-Cox8A plasmid. Labeling of transfected cells with a fluorescent SNAP-Cell substrate should show strong mitochondrial fluorescence. In most instances, difficulties in expression can be resolved by altering the transfection protocol.