Expression of SNAP Fusions in pSNAP-ADRB2 (N9178)

Overview

Protocol

1. Transient Expression

   Expression of the fusion protein cloned in pSNAP-ADRB2 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-ADRB2 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-ADRB2can 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-ADRB2 from the pSNAP-ADRB2 plasmid. Labeling of transfected cells with a fluorescent SNAP-Cell or SNAP-Surface substrate should show strong cell surface fluorescence. In most instances, difficulties in expression can be resolved by altering the transfection protocol.