Protocol for Cellular Labeling with SNAP-Cell® Oregon Green® (NEB #S9104)

Materials Required but not Supplied

SNAP-Cell® Oregon Green®

- Cells expressing SNAP-tag fusion proteins
- Tissue culture materials and media
- Transfection reagents
- Fluorescence microscope with suitable filter set
- 100% Molecular Biology grade DMSO
- SNAP-Cell Block (NEB #S9106) (optional)
- Anti-SNAP-tag Antibody (NEB #P9310) (optional)

Overview

SNAP-tag® fusion proteins can be expressed by transient or by stable transfection. SNAP-Cell® Oregon Green® is a green fluorescent substrate that can be used to label SNAP-tag fusion proteins inside living cells or in vitro. For the expression of fusion proteins with SNAP-tag, refer to the instructions supplied with the SNAP-tag plasmids. For cell culture and transfection methods, refer to established protocols.

Before you begin

Prepare a stock solution by dissolving one vial of SNAP-tag substrate (50 nmol) in 50 µl of DMSO to yield a labeling stock solution of 1 mM SNAP-tag substrate. Mix by vortexing for 1 minute until all the SNAP-tag substrate is dissolved. Store this stock solution in the dark at 4°C, or for extended storage at -20°C. Different stock concentrations can be made, depending on your requirements. The substrate is soluble up to at least 10 mM.

Protocol

1. Dilute the labeling stock solution 1:200 in medium to yield a labeling medium of 5 µM dye substrate. Mix dye with medium thoroughly by pipetting up and down several times (necessary for reducing background). For best performance, add the SNAP-tag substrate to complete medium, including serum (0.5% BSA can be used for experiments carried out in serum-free media). Do not prepare more medium with SNAP-tag substrate than you will consume within one hour.

2. Replace the medium on the cells expressing a SNAP-tag fusion protein with the SNAP-tag labeling medium and incubate at 37°C, 5% CO2 for 30 minutes.

Number of wells in plate	Recommended Volume for Cell Labeling*
6	1 ml
12	500 µl

24	250 µl
48	100 µl
96	50 µl

These recommendations are for culturing cells in polystyrene plates. For confocal imaging, use chambered coverglass, i.e Nunc® Lab-Tek II Chambered Coverglass which is available in 1, 2, 4, or 8 well formats.

3. Wash the cells three times with tissue culture medium with serum and incubate in fresh medium for 30 minutes. Replace the medium one more time to remove unreacted SNAP-tag substrate that has diffused out of the cells.

4. Image the cells using an appropriate filter set. SNAP-tag fusion proteins labeled with SNAP-Cell Oregon Green should have an excitation maximum at 490 nm and an emission maximum at 514 nm and can be imaged with standard fluorescein filter sets.

   Routinely label one well of non-transfected or mock-transfected cells as a negative control.

General Guidelines

1. Blocking Unreacted SNAP-tag® with SNAP-Cell™ Block:
In many cases the labeling of a non-transfected cell sample or a mock-transfected cell sample will be completely sufficient as a control. In some cases, however, it may be desirable to block the SNAP-tag activity in a cell sample expressing the SNAP-tag fusion protein to generate a control. This can be achieved using a nonfluorescent SNAP-tag substrate, SNAP-Cell Block™ (bromothenylpteridine, BTP). SNAP-Cell Block may also be used in pulse-chase experiments to block the SNAP-tag reactivity during the chase between two pulse-labeling steps. A protocol for blocking is included with SNAP-Cell Block (NEB #S9106).

2. Optimizing Labeling:
Optimal substrate concentrations and reaction times range from 1–10 µM and 15–60 minutes, respectively, depending on experimental conditions and expression levels of the SNAP-tag fusion protein. Best results are usually obtained at concentrations between 1 and 5 µM substrate and 30 minutes reaction time. Increasing substrate concentration and reaction time usually results in a higher background and does not necessarily increase the signal to background ratio.

3. Stability of Signal:
The turnover rates of the SNAP-tag fusion protein under investigation may vary widely depending on the fusion partner. We have seen half-life values ranging from less than one hour to more than 12 hours. Where protein turnover is rapid, we recommend analyzing the cells under the microscope immediately after the labeling reaction or, if the application allows it, fixing the cells directly after labeling. As an alternative to visualize proteins with fast turnover rates, SNAP-tag fusion proteins can be labeled at lower temperatures (4 or 16°C). Labeling times may need to be optimized.

4. Fixation of Cells:
After labeling the SNAP-tag fusion proteins, the cells can be fixed with standard fixation methods such as paraformaldehyde, ethanol, methanol, methanol/acetone, etc., without loss of signal. We are not aware of any incompatibility of the SNAP-tag label with any fixation method.

5. Counterstaining:
Cells can be counterstained with any live-cell dye that is compatible with the fluorescent properties of the SNAP-tag substrate for simultaneous microscopic detection. We routinely add 5 µM Hoechst 33342 to the medium for 2 minutes prior to completing the wash steps. Counterstaining of cells is also possible after fixation and permeabilization.

6. Immunocytochemistry:

Antibody labeling can be performed after SNAP-tag labeling and fixation of the cells according to standard protocols without loss of the SNAP-tag signal. The fixation conditions should be selected based on experience with the protein of interest. For example, some fixation methods destroy epitopes of certain proteins and therefore do not allow antibody staining afterwards.

Troubleshooting

1. No Labeling:
If no labeling is seen, the most likely explanation is that the fusion protein is not expressed. Verify your transfection method to confirm that the cells contain the fusion gene of interest. If this is confirmed, check for expression of the SNAP-tag fusion protein via Western blot. If no antibody against the fusion partner is available, Anti-SNAP-tag Antibody (NEB #P9310) can be used. Alternatively, any fluorescent SNAP-tag substrate can be used to confirm the presence of SNAP-tag fusion in cell extracts following SDS-PAGE, without the need for Western blotting.

2. Weak Labeling:
Weak labeling may be caused by insufficient exposure of the fusion protein to the substrate. Try increasing the concentration of SNAP-tag substrate and/or the incubation time, following the guidelines described above. Alternatively, the protein may be poorly expressed and/or turn over rapidly. If the protein has limited stability in the cell, it may help to analyze the samples immediately after labeling.

3. High Background:
Background fluorescence may be controlled by reducing the concentration of SNAP-tag substrate used, and by shortening the incubation time. The presence of fetal calf serum or BSA during the labeling incubation should reduce non-specific binding of substrate to surfaces.

4. Signal Strongly Reduced After Short Time:
If the fluorescence signal decreases rapidly, it may be due to instability of the fusion protein. The signal may be stabilized by fixing the cells. Alternatively try switching the SNAP-tag from the N- to the C-terminus or vice versa.

Photobleaching is generally not a problem as the SNAP-Cell Oregon Green substrate is very photostable. However, if you experience problems with photobleaching, the addition of a commercially available anti-fade reagent may be helpful.

Related Resources

- Comparison of SNAP-tag®/CLIP-tag™ Technologies to GFP
- Labeling with SNAP-tag® Technology Troubleshooting Guide
- Blocking Agents