SNAP-Cell® Fluorescein

Figure 1. Structure of SNAP-Cell Fluorescein (MW 712.7 g/mol)

Figure 2. Excitation (dotted line) and emission spectra of SNAP-Cell Fluorescein (fluorescent product of SNAP-Cell Fluorescein in cells) coupled to SNAP-tag in buffer at pH 7.5.

Materials Required but not Supplied:
- Cells expressing SNAP-tag fusion proteins
- Tissue culture materials and media
- Transfection reagents
- Fluorescence microscope with suitable filter set
- DMSO

Protocol for Labeling Reaction:
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 10 times (necessary for reducing backgrounds). 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 at 37°C, 5% CO2, for 30 minutes.

3. Wash the cells three times with tissue culture medium with serum and incubate them 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 Fluorescein should have an excitation maximum at 500 nm and an emission maximum at 532 nm, and can be imaged with standard fluorescence filter sets. We recommend routinely labeling one well of non-transfected or mock-transfected cells as a negative control.

Notes
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-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).

Optimizing Labeling
Optimal substrate concentrations and reaction times range from 1–10 µM and 15–60 minutes, respectively, depending on experimental conditions.
Stability of Signal
The turnover rates of the SNAP-tag fusion protein under investigation may vary widely depending on the fixation 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.

Fixation of Cells
After labeling the SNAP-tag fusion proteins, the cells can be fixed with standard fixation methods such as para-formaldehyde, 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.

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 prior to the first microscopic detection. We routinely add 5 µM Hoechst 33342 to the medium prior to the first microscopic detection. We routinely add 5 µM Hoechst 33342 to the medium prior to the first microscopic detection. We routinely add 5 µM Hoechst 33342 to the medium prior to the first microscopic detection.

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.

Signal Strongly Reduced After Short Time
Fluorescein has only limited photostability. Plan your experimental protocol accordingly. Minimize the cells’ exposure to light during and after labeling and to the excitation light. If you experience problems with photobleaching when working with labeled fixed cells, addition of a commercially available anti-fade reagent may be helpful. If the fluorescent signal decreases rapidly, it could also 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.

Notes for Labeling in vitro
We recommend the routine addition of 1 mM DTT to all buffers used for handling, labeling and storage of the SNAP-tag. The stability of the SNAP-tag is improved in the presence of reducing agents; however it can also be labeled in their absence, if handling at temperatures above 4°C is minimized.

SNAP-tag fusion proteins can be purified before labeling, but the labeling reaction also works in non-purified protein solutions (including cell lysates).

Troubleshooting for Labeling in vitro

Solubility
If solubility problems occur with your SNAP-tag fusion protein, we recommend testing a range of pH (pH 5.0–pH 10.0) and ionic strengths. The salt concentration may also need to be optimized for your particular fusion protein (50–250 mM).

Loss of Protein Due to Aggregation or Sticking to Tube
If stickiness of the fusion protein is a problem we recommend adding Tween 20 at a final concentration of 0.05% to 0.1%. The SNAP-tag activity is not affected by this concentration of Tween 20.