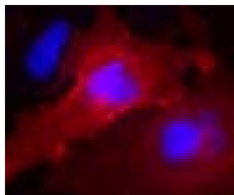
SNAP-Surface™ 549

1-800-632-7799
info@neb.com
www.neb.com

S9112S

50 nmol	Lot: 0041104
Store at: –20°C	Exp: 4/14



Live CHO-K1 cells transiently transfected with pSNAPm-ADRβ2. Cells were labeled with SNAP-Surface 549 (red) for 15 minutes and counterstained with Hoechst 33342 (blue).

Introduction

SNAP-Surface™ 549 is a photostable fluorescent substrate that can be used to label SNAP-tag® fusion proteins in solution or on the surface of living cells. This cell impermeable substrate (BG-549) is based on the Dyomics dye DY-549 and is suitable for use with standard TAMRA or Cy3 filter sets. It has an excitation maximum at 560 nm and emission maximum at 595 nm. This package includes 50 nmol of SNAP-Surface 549, sufficient to make 10 ml of a 5 µM SNAP-tag fusion protein labeling solution.

The SNAP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag is a polypeptide based on mammalian O^6-alkylguanine-DNA-alkyltransferase (AGT). SNAP-tag substrates are derivates of benzyl purines and benzyl pyrimidines. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag.

There are two steps to using this system: sub-cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice. Expression of SNAP-tag fusion proteins is described in the documentation supplied with SNAP-tag plasmids. The labeling of the fusion proteins with the SNAP-tag substrate is described below.

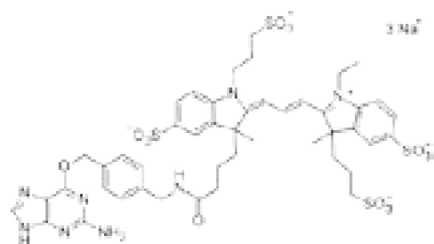


Figure 1. *Structure of BG-549 (MW 1137.2)*

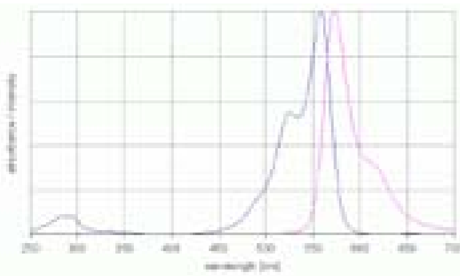


Figure 2. *Excitation (dotted line) and emission spectra of BG-549 coupled to SNAP-tag in buffer at pH 7.5*

Materials Required but not Supplied:

Cells expressing SNAP-Fusion proteins. Proteins of interest can be expressed with the SNAP-tag as either an N- or a C-terminal fusion, but note that the tag needs to be exposed to the extracellular surface of the plasma membrane for labeling with SNAP-Surface 549.

Tissue culture materials and media

Fluorescence microscope with suitable filter sets

Transfection reagents

DMSO

Storage

SNAP-Surface 549 should be stored at –20°C (long term) or at 4°C in the dark (short term, less than 4 weeks). Protect the substrate from light and moisture. With proper storage at –20°C the substrate should be stable for at least two years dry or 3 months dissolved in DMSO.

Quality Controls

Purity and Characterization: Purity of SNAP-Surface 549 was determined to be 96% by HPLC analysis. Molecular weight $[M]^-$ was determined by MS to be 1069.3 (1069.3 expected).

***In vitro* Protein Labeling:** Reaction of SNAP-Surface 549 (10 µM) with purified SNAP-tag protein (5 µM) *in vitro*, followed by mass spec analysis, indicated an efficiency of labeling of 95%.

Cellular Protein Labeling: Cells transfected with SNAP-tag vectors expressing ADRβ2 (cell surface) and H2B (nucleus) were labeled with 5 µM SNAP-Surface 549 for 30 minutes, and visualized by confocal microscopy. Only surface target was efficiently labeled.

Instructions for Cellular Labeling

SNAP-tag fusion proteins can be expressed by transient or by stable transfection. For expression of fusion proteins with the SNAP-tag refer to instructions supplied with the SNAP-tag plasmids. For cell culture and transfection methods, refer to established protocols.

Dissolve 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 for 10 minutes 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 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 clean 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 mediumand incubate at 37°C, 5% CO_2 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, we recommend using chambered coverglass such as Lab-Tek II Chambered Coverglass which is available in a 1, 2, 4 or 8 well format from Nunc (www.nuncbrand.com).

3. Wash the cells three times with tissue culture medium with serum.

4. Image the cells using an appropriate filter set. SNAP-tag fusion proteins labeled with SNAP-Surface 549 should have an excitation maximum at 560 nm and an emission maximum at 595 nm, and can be imaged with Cy3 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-Surface™ 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 cell-impermeable SNAP-tag substrate, SNAP-Surface Block (C8-propanoic acid benzylguanine, CBG). SNAP-Surface 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-Surface Block (NEB #S9143).

Optimizing Labeling

Optimal substrate concentrations and reaction times range from 5–10 µM and 5–15 minutes, respectively, depending on experimental conditions and expression levels of the SNAP-tag fusion protein. Increasing substrate concentration and reaction time usually results in a higher background and does not necessarily increase the signal to background ratio.

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.

(see other side)

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, ethanol/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 that is used for the final 30 minutes incubation (Step 3) as a DNA counterstain for nuclear visualization. Counterstaining of cells is also possible after fixation and permeabilization.

Immunocytochemistry

Antibody labeling after SNAP-tag labeling and fixation of the cells should be possible 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

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. If no antibody against the fusion partner is available, a commercial anti-AGT antibody can be used (Chemicon, mouse Anti-MGMT [O^6-methylguanine-DNA methyltransferase] Monoclonal Antibody, Clone MT3.1 Catalog number MAB16200). Alternatively, SNAP-Vista Green (NEB #S9147) can be used to confirm presence of SNAP-tag fusion in cell extracts following SDS-PAGE, without the need for Western blotting.

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.

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

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 not generally a problem as the SNAP-Surface 549 substrate is very photostable. However, if you experience problems with photobleaching, addition of a commercially available anti-fade reagent may be helpful.

Instructions for Labeling Proteins *in vitro*

Dissolve the vial of SNAP-tag substrate (50 nmol) in 17 μl of DMSO to yield a stock solution of 3 mM SNAP-tag substrate. Pipette up and down periodically for 10 minutes until all the SNAP-tag substrate is dissolved.

Protocol for Labeling Reaction:

1. Prepare a protein solution containing up to 20 μM SNAP-tag fusion protein to be labeled in an appropriate buffer containing at least 1 mM DTT.

2. Add SNAP-tag substrate solution to a total volume of 1% of the volume of the protein solution. Carefully pipette the material up and down to mix, and vortex briefly.

3. Incubate for 1 hour at 25°C in the dark. Alternatively incubate overnight at 4°C in the dark.

Removal of Unreacted Substrate (optional)

After the labeling reaction the unreacted substrate can be separated from the labeled SNAP-tag fusion protein by gel filtration or dialysis. Please refer to the vendor's instructions for the separation tools you are using.

Notes for Labeling in Solution

We recommend the routine addition of 1 mM DTT to all buffers for 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.

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

Troubleshooting

Labeling Reaction

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).

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.

If exhaustive labeling of a protein sample is not achieved using the recommended conditions, try the following protocol modifications: Double the incubation time to two hours total at 25°C or to 24 hours at 4°C; or halve the volume of protein solution labeled (50 μl of a solution containing up to 20 μM SNAP-tag fusion protein). Both approaches may be combined. If you still have poor labeling results, we recommend checking the activity of the SNAP-tag using SNAP-Vista.

If your fusion protein is particularly sensitive to degradation or to loss of activity, you can try reducing the labeling time or decreasing the labeling temperature. If you label at 4°C we recommend over night incubation.

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