SNAP-Cell® 505-Star

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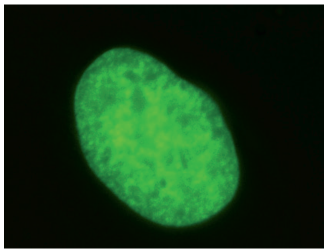
S9103S 005151018101

S9103S

50 nmol	**Lot: 0051510**
Store at: –20°C	**Exp: 10/18**



Live U-2 OS cells stably transfected with pSNAP$_f$-H2B. Cells were labeled with SNAP-Cell 505-Star (green) for 15 minutes at 37°C, 5% CO_2.

Introduction

SNAP-Cell 505-Star is a photostable green fluorescent substrate that can be used to label SNAP-tag® fusion proteins inside living cells, on cell surfaces, or *in vitro*. This cell-permeable substrate (CP-6-505) is based on the single isomer 6-carboxyrhodamine 110 fluorescent dye and is suitable for standard fluorescein filter sets. It has an excitation maximum at 504 nm and an emission maximum at 532 nm. This package contains 50 nmol of SNAP-Cell 505-Star substrate, 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 based on the mammalian O^6-alkylguanine-DNA-alkyltransferase (AGT). SNAP-tag substrates are derivatives of benzylguanines and benzylchloropyrimidines. 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: subcloning 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.

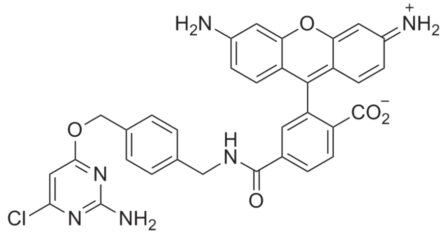


Figure 1. Structure of SNAP-Cell 505-Star (MW 621.0 g/mol).

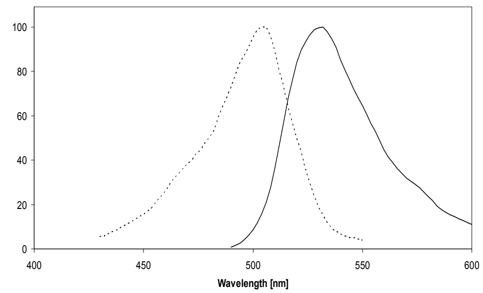


Figure 2. Excitation (dotted line) and emission spectra of SNAP-Cell 505-Star coupled to SNAP-tag in buffer at pH 7.5

The labeling of the fusion proteins with the SNAP-tag substrate is described below.

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

Storage

SNAP-Cell 505-Star 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 three years dry or 3 months dissolved in DMSO.

Quality Controls

Purity and Characterization: Purity of SNAP-Cell 505-Star was determined to be 98% by HPLC analysis. Molecular weight $[M+H]^+$ was determined by MS to be 620.1 (620.2 expected).

***In vitro* protein Labeling:** Reaction of SNAP-Cell 505-Star (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 Histone H2B (intracellular) were labeled in separate experiments with 5 µM SNAP-Cell 505-Star for 30 minutes and visualized by epifluorescent microscopy. The intracellular 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 by vortexing 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 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 and 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

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 505-Star should have an excitation maximum at 504 nm and an emission maximum at 532 nm, and can be imaged with standard fluorescein 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-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).

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.

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)

CERTIFICATE OF ANALYSIS

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 wash step (Step 3) as a DNA counterstain for nuclear visualization and leave this on the cells for 2 minutes prior to completing the wash steps. Counterstaining of cells is also possible after fixation and permeabilization.

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 for Cellular Labeling

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, Anti-SNAP-tag Antibody (NEB #P9310) can be used. Alternatively, SNAP-Vista® Green (NEB #S9147) can be used to confirm the 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 generally not a problem as the SNAP-Cell 505-Star 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 of Proteins *in vitro*:

1. Dissolve the vial of SNAP-Cell 505-Star (50 nmol) in 50 µl of fresh DMSO to yield a labeling stock solution of 1 mM SNAP-tag substrate. Mix by vortexing for 10 minutes until all the SNAP-tag substrate is dissolved. Dilute this 1 mM stock solution 1:4 in fresh DMSO to yield a 250 µM stock for labeling proteins *in vitro.*

2. Set up the reactions, in order, as follows:

Component	Volume	Final Concentration
Phosphate Buffered Saline (PBS)	42 µl	1X
50 mM DTT	1 µl	1 mM
50 µM SNAP-tag Purified Protein	5 µl	5 µM
250 µM SNAP-tag Substrate	2 µl	10 µM
Total Volume	50 µl	

3. Incubate in the dark for 30 minutes at 37°C.

4. Run sample on an SDS-PAGE gel and detect using a fluorescent gel scanner or store samples at –20°C or –80°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 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.

Incomplete Labeling

If exhaustive labeling of a protein sample is not achieved using the recommended conditions, try the following protocol modifications: Increase 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. Both approaches may be combined. If you still have poor labeling results, we recommend checking the activity of the SNAP-tag using SNAP-Vista Green.

If the SNAP-tag fusion has been stored in the absence of DTT or other reducing agent, or has been stored at 4°C for a prolonged period, its activity may be compromised. Include 1 mM DTT in all solutions of the SNAP-tag fusion protein, and store the fusion protein at –20°C.

Using less than the recommended amount of substrate stock solution can significantly slow down the reaction rate.

Loss of Activity of Protein of Interest

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 overnight incubation.

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This product is covered by US Patent 8,178,314.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

The products and/or their use may be covered by one or more of the following patents and patent applications: U.S. Patent No. 7,939,284 (Methods for Using O6-Alkylguanine-DNA-Alkyltransferases); U.S. Patent No. 7,888,090 (Mutants of O6-Alkylguanine-DNA-Alkyltransferases); U.S Patent No. 8,163,479 (Specific Substrates for O6-Alkylguanine-DNA-Alkyltransferases); U.S. Patent No. 8,178,314 (Pyrimidines reacting with O6-Alkylguanine-DNA-Alkyltransferases); PCT/EP2007/057597 (Labeling of Fusion Proteins with Synthetic Probes); EP07117800 (Drug Delivery); EP07117802 (Drug Delivery); EP07120288 (GTPase-Transient Protein Protein Interactions) These patents and patent applications are owned by Covalys, or owned by the Ecole Polytechnique Fédérale de Lausanne (EPFL) and exclusively licensed to Covalys and NEB.