SNAP-Biotin

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S9110S 002120815081

S9110S

50 nmol **Lot: 0021208**

Store at: –20°C **Exp: 8/15**

Introduction

SNAP-Biotin is a cell-permeable substrate (BG-Biotin) based on biotin with an amidocaproyl linker. It is suitable for applications such as biotinylation of SNAP-tag® fusion proteins in living cells for detection with streptavidin fluorophore conjugates or labeling in solution for analysis by SDS-PAGE/Western Blot or for capture with streptavidin for binding and interaction studies. This package contains 50 nmol of SNAP-Biotin substrate, sufficient to make 10 ml of a 5 µM solution for the labeling of SNAP-tag fusion proteins in cells.

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 small polypeptide based on mammalian O^6-alkylguanine-DNA-alkyltransferase (AGT). SNAP-tag substrates are derivatives of benzyl purines and benzyl pyrimidines. In the labeling reaction, the substituted benzyl group of the substrate becomes 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. The labeling of fusion proteins with the SNAP-Biotin substrate is described below.

Materials required but not supplied:

Cells expressing SNAP-tag proteins
Tissue culture materials and media
Transfection reagents
DMSO

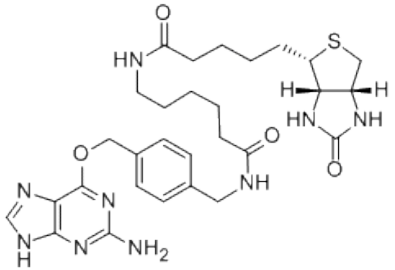

Figure 1. *Structure of SNAP-Biotin (MW 609.7 g/mol)*

Storage

SNAP-Biotin should be stored at –20°C (long term) or at 4°C (short term, less than 4 weeks). 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-Biotin was determined to be 99% by HPLC analysis. Molecular weight $[M]^-$ was determined by MS to be 608.3 (608.3 expected).

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

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 and the guidelines from your expression plasmid provider. 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 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 biotin substrate. Mix substrate 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

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 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.

   We recommend routinely labeling one well of non-transfected or mock-transfected cells as a negative control.

Microscopy

Fixation: After labeling the SNAP-tag fusion proteins with SNAP-Biotin, the cells can be fixed with 3.3% para-formaldehyde which does not result in the loss of signal. Avoid fixation using ethanol as this may lead to a high background staining of endogenous biotinylated proteins found preferentially in mitochondria.

Detection: To visualize the SNAP-tag fusion protein *in situ*, permeabilize the cells with 0.5% Triton in PBS and block the cells with 1% BSA in PBS containing 0.5% Triton. Incubate the fixed cells with an appropriate streptavidin/avidin conjugate (e.g. streptavidin-fluorophore) and image the cells according to the instructions supplied with the conjugate.

Western Blotting

Biotinylated proteins from cell lysates can be visualized on Western Blots using standard streptavidin-based detection reagents. For Western blotting experiments, it may be more efficient to label the SNAP-tag fusion proteins after lysis of the cells in the lysate. One may also use the Anti-SNAP-tag Antibody (NEB #P9310).

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 in live cells 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 processing the cells for imaging or blotting immediately after the labeling reaction.

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.

(see other side)

CERTIFICATE OF ANALYSIS

Immunocytochemistry

Antibody labeling of the fusion protein 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 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. Improving the protein expression may also improve the signal. If the protein has limited stability in the cell, it may help to analyze the samples immediately after labeling.

Instructions for Labeling of Proteins *in vitro*:

1. Dissolve the vial of SNAP-Biotin (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
Deionized Water	32 µl	
5X SNAP-tag Reaction Buffer	10 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 standard streptavidin-based detection reagents 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).

Confirmation of Labeling by Western blot Analysis

Labeled SNAP-tag fusion proteins can be easily analyzed on a SDS-PAGE gel/Western blot analysis because the covalently bound label will remain attached to the protein. The biotin label can be detected on an SDS-PAGE gel followed by Western blot using a Horseradish Peroxidase or Alkaline Phosphatase labeled avidin/streptavidin (e.g. streptavidin-HRP) and the corresponding detection method as described by the supplier of the enyzme conjugate.

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.