**SNAP-Surface® Block**

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S9143S 007150318031

# S9143S

**200 nmol** **Lot: 0071503**

**Store at –20°C** **Exp: 3/18**

### Introduction

SNAP-Surface Block (C8 propanoic acid benzylguanine, CBG) is a nonfluorescent compound that blocks the reactivity of the SNAP-tag® in solution or on the surface of living cells. It can be used to generate inactive controls in live cell labeling experiments performed with SNAP-tag fusion proteins. SNAP-Surface Block reacts with the SNAP-tag irreversibly, inactivating it for subsequent labeling steps. This blocker is largely membrane impermeable essentially limiting blocking to cell surface-exposed SNAP-tags.

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 protein based on mammalian $O^6$-alkylguanine-DNA-alkyltransferase (AGT). SNAP-tag substrates are derivatives of benzylpurines 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 instructions supplied with SNAP-tag plasmids. The labeling of SNAP-tag fusion proteins with SNAP-Surface substrates is described in the instructions supplied with SNAP-Surface substrates. The use of SNAP-Surface Block during the labeling of fusion proteins with SNAP-Surface substrates is described below.

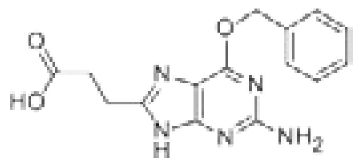


***Figure 1.*** *Structure of SNAP-Surface Block (MW 313.3 g/mol)*

### 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-Surface Block should be stored at –20°C (long term) or at 4°C (short term). With proper storage at –20°C, SNAP-Surface Block is stable for at least three years dry or 3 months when dissolved in DMSO.

### Quality Controls

**Purity and Characterization:** Purity of SNAP-Surface Block was determined to be 99% by HPLC analysis. Molecular weight $[M+H]^+$ was determined by MS to be 314.1. (314.1 expected).

***In vitro* protein labeling:** Reaction of SNAP-Surface Block (20 µM) with purified SNAP-tag protein (5 µM) *in vitro*, followed by mass spec analysis, indicated an efficiency of labeling of ≥ 95%.

**Blocking of cellular protein labeling:** Cells transfected with SNAP-tag vector expressing ADRβ2 were reacted sequentially with 20 µM SNAP-Surface Block and 5 µM SNAP-Surface™ 549 for 30 minutes, and visualized by confocal microscopy. No labeling was detected.

### Instructions for use with SNAP-Surface substrates

In many cases the labeling of a non-transfected cell sample or a mock-transfected cell sample will be completely sufficient as a negative control for cell labeling. In some cases, however, it may be desirable to selectively block the SNAP-tag activity on the cell-surface in a cell sample expressing the SNAP-tag fusion protein to generate a control. This is done by a pre-incubation of the cells with SNAP-Surface Block, followed by the incubation with the labeling solution. 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.

**Note:** SNAP-Surface Block should block > 90% of active SNAP-tag on the cell surface under the conditions given below, however complete blocking is difficult to achieve. SNAP-Surface Block is slightly cell permeable so use of it may slightly reduce signal from intracellular tagged proteins in later labeling steps. This effect is increased when SNAP-Surface Block is used at higher concentrations and for longer incubation times. Always take care to avoid carryover of SNAP-Surface Block to samples that you do not wish to block.

The following steps describe the use of SNAP-Surface Block in a typical labeling experiment:

1. Dissolve one vial of SNAP-Surface Block (200 nmol) by adding 50 µl of DMSO to give a solution of 4 mM SNAP-Surface Block. Mix by vortexing for 10 minutes, until all the SNAP-Surface Block is dissolved. Store this stock solution in the dark at 4°C or for extended storage at –20°C. We recommend using a final concentration of 20 µM, which is a 1:200 dilution of this stock solution.

2. Prepare two cell samples suitable for labeling, expressing the SNAP-tag fusion protein of interest.

3. Dilute the blocking stock solution 1:200 in medium to yield a blocking medium of 20 µM SNAP-Surface Block. Mix blocker with medium thoroughly by pipetting up and down 10 times. For best performance, add the dissolved SNAP-Surface Block 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-Surface Block than you will consume within one hour.

4. Mix an appropriate amount of medium with DMSO in a ratio of 1:200, to give a final concentration of 0.5% v/v DMSO. Mix thoroughly by pipetting up and down 10 times.

5. Replace the medium on one sample of cells with the blocking medium. These are your Blocked Cells. Replace the medium on the other sample of cells with the medium containing DMSO. These are your Test Cells. Incubate both cell samples for 20 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 |

6. Remove SNAP-Surface Block or DMSO-containing medium by washing both samples of cells twice with complete medium.

7. Label both cell samples with the fluorescent SNAP-Surface substrate using the supplied protocol.

8. Inspect both samples under the fluorescence microscope. The Blocked Cells should show no fluorescence, whereas the Test Cells should show fluorescence localized to where the SNAP-tag fusion protein is present in the cell.

**Note:** Please note that there is a constant turnover of proteins in the cell. Protein transport to the membrane and internalization followed by degradation or recycling, are constantly ongoing processes. After having blocked SNAP-tag on the cell membrane, newly synthesized protein may be transported to the cell surface and may get labeled during incubation with a fluorescent SNAP-tag substrate. This will give the impression that the blocking was ineffective. In order to minimize these effects of protein synthesis and protein transport, cells may have to be treated with cycloheximide and incubation with the fluorescent SNAP-tag substrate may have to be performed at 4°C.

### Instructions for Labeling of Proteins *in vitro*:

1. Dissolve the vial of SNAP-Surface Block (200 nmol) in 50 µl of fresh DMSO to yield a labeling stock solution of 4 mM SNAP-Surface Block. Mix by vortexing for 10 minutes until all the SNAP-tag substrate is dissolved. Dilute this 4 mM stock solution 1:4 in fresh DMSO to yield a 1 mM stock for labeling proteins *in vitro.*

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

| Component | Volume | Final Concentration |
|---|---|---|
| Phosphate Buffered Saline (PBS) | 40 µl | 1X |
| 50 mM DTT | 1 µl | 1 mM |
| 50 µM SNAP-tag Purified Protein | 5 µl | 5 µM |
| 1 mM SNAP-Surface Block | 2 µl | 20 µM |
| 250 µM SNAP-tag Substrate | 2 µl | 10 µM |
| **Total Volume** | 50 µl | |

3. Incubate sample containing only 20 µM SNAP-Surface Block in the dark for 20 minutes at 37°C.

*(see other side)*

CERTIFICATE OF ANALYSIS

4. Once incubation with SNAP-Surface Block is complete, add 2 µl of 250 µM SNAP-tag substrate, mix and incubate in the dark for 30 minutes at 37°C.

5 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 troubleshooting please refer to the instructions supplied with SNAP-Surface products as appropriate.

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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); EPO7120288 (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.