SNAP-Cell® Block

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S9106S 007120615061

S9106S

100 nmol **Lot: 0071206**

Store at –20°C **Exp: 6/15**

Introduction

SNAP-Cell® Block (bromothenylpteridine, BTP) is a non-fluorescent compound that blocks the reactivity of the SNAP-tag® in solution or in living cells. It can be used to generate inactive controls in live cell labeling experiments performed with SNAP-tag fusion proteins. SNAP-Cell Block is highly membrane permeable and once in the cell reacts with the SNAP-tag, irreversibly inactivating it for subsequent labeling steps.

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 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-Cell substrates is described in the instructions supplied with SNAP-Cell substrates. The use of SNAP-Cell Block during the labeling of fusion proteins with SNAP-Cell substrates is described below.

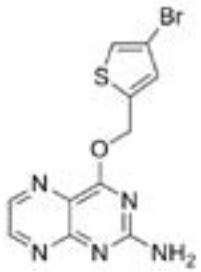

Figure 1. *Structure of SNAP-Cell Block (MW 338.2 g/mol)*

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

Quality Controls

Purity and Characterization: Purity of SNAP-Cell Block was determined to be 90% by HPLC analysis. Molecular weight $[M+H]^+$ was determined by MS to be 337.9 (338.0 expected).

***In vitro* protein labeling:** Reaction of SNAP-Cell Block (10 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 H2B-SNAP (intracellular) were reacted sequentially with 10 µM SNAP-Cell Block for 20 minutes, followed by 3 µM SNAP Cell TMR-Star for 30 minutes, and visualized by confocal microscopy. No labeling was detected.

Instructions for Use with SNAP-Cell 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 block the SNAP-tag activity 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-Cell Block, followed by the incubation with the labeling solution. 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.

Note: SNAP-Cell Block is a potent blocker of the SNAP-tag. Always take care to avoid carryover of SNAP-Cell Block to samples that you do not wish to block.

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

1. Dissolve one vial of SNAP-Cell Block (100 nmol) by adding 50 µl of DMSO to give a solution of 2 mM SNAP-Cell Block. Mix by vortexing for 10 minutes, until all the SNAP-Cell 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 10 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 10 µM SNAP-Cell Block. Mix blocker with medium thoroughly by pipetting up and down 10 times. For best performance, add the dissolved SNAP-Cell Block to complete medium, including serum (0.5% BSA can used for experiments carried out in serum-free media). Do not prepare more medium with SNAP-Cell 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

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).

6. Remove SNAP-Cell Block or DMSO-containing medium by washing both samples of cells twice with complete medium.
7. Label both cell samples with the fluorescent SNAP-Cell 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 and resynthesis of proteins in the cell. After having blocked all existing SNAP-tag fusion proteins within the cell, new SNAP-tag fusion protein molecules may be synthesized in the meantime 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-Cell Block (100 nmol) in 50 µl of fresh DMSO to yield a labeling stock solution of 2 mM SNAP-Cell Block. Mix by vortexing for 10 minutes until all the SNAP-tag substrate is dissolved. Dilute this 2 mM stock solution 1:4 in fresh DMSO to yield a 500 µM stock for labeling proteins *in vitro*.
2. Set up the reactions, in order, as follows:

Component	Volume	Final Concentration
Deionized Water	30 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
500 µM SNAP-Cell 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-Cell Block in the dark for 20 minutes at 37°C.
4. Once incubation with SNAP-Cell 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-Cell products as appropriate.