SNAP-Cell™ Oregon Green®

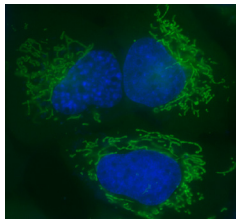
NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

S9104S 005120814081

S9104S

50 nmol **Lot: 0051208**

Store at: –20°C **Exp: 8/14**



Live U2OS cells transiently transfected with pSNAP-Cox8A (mitochondrial expression). Cells were labeled with SNAP-Cell Oregon Green (green) for 15 minutes and counterstained with Hoechst 33342 (blue).

Introduction

SNAP-Cell™ Oregon Green® is a photostable green fluorescent substrate that can be used to label SNAP-tag® fusion proteins inside living cells. This cell-permeable substrate (BG-Oregon Green) is based on the Invitrogen dye, Oregon Green® and is suitable for standard fluorescein filter sets. It has an excitation maximum at 490 nm and an emission maximum at 514 nm. Conjugates of Oregon Green are more photostable than those of fluorescein, and their fluorescence properties are essentially pH insensitive in the physiological pH range. This package contains 50 nmol of SNAP-Cell Oregon Green substrate, sufficient to make 10 ml of a 5 uM 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 small protein based on mammalian O^6-alkylguanine-DNA-alkyltransferase (AGT). SNAP-tag substrates are derivates of benzylguanine. In the labeling reaction, the dye-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 the fusion proteins with the SNAP-tag substrate is described below.

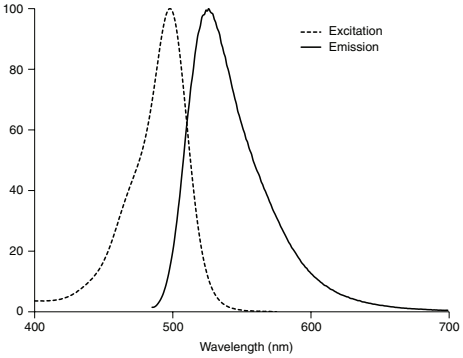


Figure 1. *Excitation (dotted line) and emission spectra after coupling of SNAP-Cell Oregon Green to SNAP-tag in buffer at pH 7.5.*

Note: SNAP-Cell Oregon Green is not suitable for labeling of cell surface SNAP-tag fusion proteins. The Oregon Green dye is based on a fluorinated fluorescein modified with pivaloyl groups which is uncharged and essentially non-fluorescent. It becomes fluorescent once inside the cell where it is hydrolyzed by non-specific esterases.

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 Oregon Green 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-Cell Oregon Green was determined to be 92% by HPLC analysis. Molecular weight $[M+H]^+$ was determined by MS to be 833.3 (833.3 expected).

***In vitro* protein Labeling:** Reaction of SNAP-Cell Oregon Green (10 µM) with purified SNAP-tag protein (5 µM) *in vitro*, for 30 minutes at 37°C, followed by mass spec analysis, indicated an efficiency of labeling of 26%.

Cellular Protein Labeling: Cells transiently transfected with SNAP-tag vectors expressing Histone 2B (intracellular) were labeled in separate experiments with 5 µM SNAP-Cell Oregon Green for 30 minutes, 37°C, 5% CO_2, and visualized by fluorescent microscopy. The Histone 2B 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 (25 nmol) in 25 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 several times (necessary for reducing background). 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.

4. Image the cells using an appropriate filter set. SNAP-tag fusion proteins labeled with SNAP-Cell Oregon Green should have an excitation maximum at 490 nm and an emission maximum at 514 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).

(see other side)

CERTIFICATE OF ANALYSIS

Optimizing Labeling

Optimal substrate concentrations and reaction times range from 2–20 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 2 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. As an alternative to visualize proteins with fast turnover rates, SNAP-tag fusion proteins can be labeled at lower temperatures (4 or 16°C). Labeling times may need to be optimized.

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 for 5 minutes prior to 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 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 via Western blot. 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 Oregon Green 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 Oregon Green (25 nmol) in 25 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	Vol.	Final Conc.
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 a fluorescent gel scanner or store samples at –20°C or –80°C in the dark.