Figure 1. Structure of SNAP-Vista Green (MW 628.6 g/mol)

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

Figure 3. Typical SDS-PAGE gel from a gel scanner (Molecular Dynamics Storm Imager; 488 nm excitation). Lane 1 fluorescent MW marker; Lane 2-4 SNAP-His (22 kD) 0.1, 0.5, 1.0 µg; Lane 5-7 SNAP-CyA (40 kD) 0.1, 0.5, 1.0 µg; Lane 8-10 MBP-SNAP (63 kD) 0.1, 0.5, 1.0 µg.

Table: Protocol for Labeling Mammalian Cell Lysates

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate Buffered Saline (PBS)</td>
<td>42 µl</td>
<td>1X</td>
</tr>
<tr>
<td>50 mM DTT</td>
<td>1 µl</td>
<td>1 mM</td>
</tr>
<tr>
<td>50 µM SNAP-tag Purified Protein</td>
<td>5 µl</td>
<td>5 µM</td>
</tr>
<tr>
<td>250 µM SNAP-tag Substrate</td>
<td>2 µl</td>
<td>10 µM</td>
</tr>
<tr>
<td>Total Volume</td>
<td>50 µl</td>
<td></td>
</tr>
</tbody>
</table>

Introduction

SNAP-Vista Green is a green fluorescent substrate that can be used to label SNAP-tag fusion proteins (in cell lysates or purified proteins) for detection by SDS-PAGE. This substrate (BG-Vista Green) is based on human O6-alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag substrates are fluorophores, biotin or beads conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag.

The SNAP-tag protein labeling system enables the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag is based on human O6-alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag substrates are fluorophores, biotin or beads conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker. 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: sub-cloning 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 for detection by SDS-PAGE is described in this document.

Instructions for Use

Protocol for Labeling Mammalian Cell Lysates

1. Harvest cells by trypsinization following established protocols.
2. Wash cells twice with PBS.
3. Lyse cells by suspending in reaction buffer at 10^10 cells per 20 µl. Reaction buffer is 50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 0.1% Tween-20, 1 mM DTT and an EDTA-free protease inhibitor cocktail (e.g., Complete“, Roche).
4. Dilute the 1 mM SNAP-Vista Green stock solution 1:4 in fresh DMSO to yield a 250 µM stock solution. Add 2 µl of the SNAP-Vista Green 250 µM stock solution to 18 µl of cell lysate. Mix well by pipetting up and down several times.
5. Incubate in the dark for 30 minutes at 37°C.
6. Add an appropriate volume of concentrated SDS-PAGE sample buffer and proceed with sample preparation and SDS-PAGE according to the gel manufacturer’s instructions.
7. After the gel is run, immediately obtain a fluorescent image using a laser scanner with 488 nm excitation or a UV-transilluminator and an appropriate camera (Polaroid or digital). Excitation at 488 nm will give the best results. The fluorescence is an intense green.
8. After fluorescent imaging, standard fixing and staining protocols can be used to detect the non-fluorescent proteins.

Notes

Most gel fixing/staining protocols will affect the fluorescence of the SNAP-Vista Green substrate. The fluorescent gel image should be appropriately documented before continuing with protein staining.

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.

(see other side)
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 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.