SNAP-Vista Green

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S9147S 001120615061

S9147S

50 nmol **Lot: 0011206**

Store at: –20°C **Exp: 6/15**

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 fluorescein and is optimized for excitation with the 488 nm laser excitation line in a laser based gel scanner. It can also be excited using 360 nm light from a standard UV-transilluminator. It has an excitation maximum at 500 nm and emission maxima at 524 nm. This package includes 50 nmol of SNAP-Vista Green substrate, sufficient to label one hundred 20 µl samples containing SNAP-tag fusion protein for in-gel detection.

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 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 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.

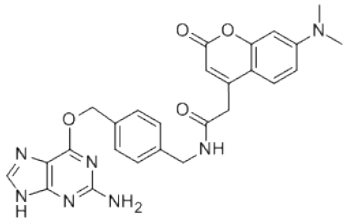

Figure 1. *Structure of BG-Vista Green (MW 628.6)*

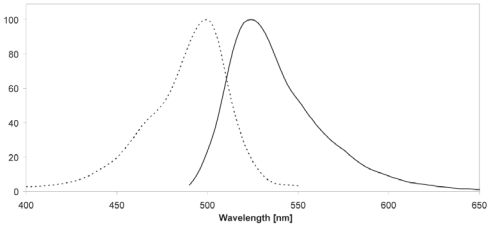

Figure 2. *Excitation (dotted line) and emission spectra of BG-vista Green coupled to SNAP-tag in buffer at pH 7.5*

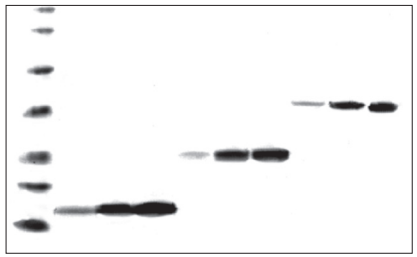

Figure 3. *Typical SDS-PAGE gel from a gel scanner (Molecular Dynamics Storm Imager, 488 nm excitation). Lane 1 fluorescent MW markers (Sigma P/N F3526); Lane 2-4 SNAP-His (22 kD) 0.1, 0.5, 1.0 µg; Lane 5-7 SNAP-CyA (40 kD) 0.1, 0.5, 1.0 µg; Lane 8-10 MBP-SNAP (63 kD) 0.1, 0.5, 1.0 µg.*

Storage

Store substrate at –20°C. After dissolving SNAP-Vista Green store it in aliquots at –20°C in the dark for up to three months. Once an aliquot is thawed it can be stored at 4°C in the dark for up to two weeks. Make sure substrate, which may precipitate during freezing, is completely re-dissolved before use.

To make a stock solution, dissolve substrate in 200 µl of a general purpose protein buffer containing 1 mM DTT (e.g., 50 mM Tris, 100 mM NaCl, 0.1% Tween 20, 1 mM DTT, pH 7.5–8.0). Vortex twice for 30 seconds or until all substrate is dissolved. Dispense into 50 µl aliquots for storage.

Quality Controls

Purity and Characterization: Purity of SNAP-Vista Green was determined to be 99% by HPLC analysis. Molecular weight $[M+H]^+$ was determined by MS to be 629.2 (629.2 expected).

***In vitro* Protein Labeling:** Reaction of SNAP-Vista Green (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 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 10^4–10^5 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. Add 2 µl of the SNAP-Vista stock solution to 18 µl of cell lysate. Mix well by pipetting up and down several times.
5. Incubate in the dark for 20 minutes at room temperature.
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.

Protocol for Labeling Proteins *in vitro*:

1. Add 2 µl of the substrate stock solution to 18 µl of protein sample containing a SNAP-tag fusion protein in an appropriate buffer (see notes). Mix well by pipetting up and down several times.
2. Incubate in the dark for 20 minutes at room temperature.
3. 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.
4. After the gel is run, immediately take 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.
5. After fluorescent imaging, standard fixing and staining protocols can be used to detect the non-fluorescent proteins.

Notes

SNAP-Vista Green is dried down with mannitol to improve its aqueous solubility. Mannitol will be present at 10 mM final concentration and should not lead to any problems.

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

We recommend the routine addition of 1 mM DTT to all buffers for used for handling, labeling and storage of the SNAP-tag. This will enhance the labeling by improving the stability and reactivity of the SNAP-tag fusion protein. Labeling also works under non-reducing conditions. Care should be taken to avoid handling the SNAP-tag fusion protein above 4°C prior to labeling.

Where stickiness of the fusion protein is a problem we recommend adding Tween 20 at a final concentration of 0.05% to 0.1%. At this concentration Tween 20 does not affect the performance of the SNAP-tag.

Correct storage and handling of unlabeled SNAP-tag fusion proteins is essential to maintain reactivity of the SNAP-tag prior to labeling. Unlabeled fusion proteins should be stored at –20°C and thawed just before use. Prolonged handling at temperatures above 4°C should be avoided, especially if the protein is stored in the absence of reducing agents (e.g., DTT).

The SNAP-tag labeling reaction is tolerant of a wide range of buffers. The requirements of the fusion partner should dictate the buffer selected. The following buffer guidelines are recommended: pH between 7.0 and 8.0, monovalent salts (e.g. sodium chloride) between 50 mM and 250 mM, at least 1 mM DTT. Non-ionic detergents can be added to 0.5% v/v if required, but SDS and other ionic detergents should be avoided entirely because they inhibit the activity of the SNAP-tag. Metal chelating reagents (e.g., EDTA and EGTA) also inhibit SNAP-tag activity and should be avoided. Many proteins benefit from the addition of glycerol for frozen storage, typically 20% v/v.

(see other side)

Unreacted SNAP-vista substrate will run in front of the protein bands in the gel, running at an equivalent molecular weight below 20 kDa (below the band obtained for SNAP-tag alone). If the fluorescence from the unreacted substrate interferes with imaging for your protein, you may separate the labeled protein from unreacted substrate after the labeling reaction and before running the gel using, for example, a spin separation device.

Troubleshooting

Labeling Reaction

If solubility problems occur, we recommend testing a range of pH (pH 7.0–pH 8.0) and ionic strengths. The salt concentration (50–250 mM) may also need to be optimized for your particular fusion protein.

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.