BG-GLA-NHS

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S9151S 022151217121

S9151S

2 mg **Lot: 0221512**

Store at:–20°C **Exp: 12/17**

Introduction

BG-GLA-NHS is an amine-reactive building block for the one-step synthesis of SNAP-tag® substrates from amine-containing precursors including proteins, peptides or oligonucleotides. This building block allows you to make custom SNAP-tag substrates for labeling SNAP-tag fusion proteins.

The SNAP-tag protein labeling system enables the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag is a protein based on human O^6-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: 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 in this document.

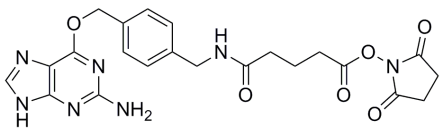

Figure 1. *Structure of BG-GLA-NHS (MW 481.5 g/mol)*

Storage

BG-GLA-NHS is supplied in lyophilized form and packaged under argon. The dry SNAP-tag building block substrate should be stored at –20°C.

BG-GLA-NHS is soluble in anhydrous DMF or DMSO. Choose a solvent volume that will be compatible with the final use. Vortex and centrifuge briefly to remove any non-dissolved solids.

NHS esters readily hydrolyze in moist environments. Experiments should be designed to maximize the usage of BG-GLA-NHS. Storage of unused BG-GLA-NHS solubilized in DMF or DMSO should be avoided. If unavoidable, we recommend storing at –20°C for the minimum time period.

Quality Control

Characterization: Purity of BG-GLA-NHS was determined to be 90% by HPLC analysis. Molecular weight $[M+H]^+$ was determined by MS to be 482.1 (482.2 expected)

Reaction Conditions for Chemical Coupling

Coupling BG-GLA-NHS to Proteins

NHS coupling to proteins is often performed using an excess of NHS ester. Depending on the number of lysine residues in the protein, it may be necessary to stop the labeling reaction before it reaches completion to avoid damaging the protein or causing it to unfold. A typical coupling involves reacting the NHS ester with exposed primary amines on the surface of the proteins to form an amide bond. The reaction can be carried out at pH 7-9 (typically pH 8.3), temperatures from 4–37°C and incubation times ranging from 30 min to overnight. Care must be taken to avoid primary amine containing buffers such as Tris or ammonium ions.

Coupling BG-GLA-NHS to Amine-Containing Compounds

A typical procedure involves equimolar quantities of NHS ester, amine to be coupled, and triethylamine in DMF at 30°C overnight. The purification strategy will depend on the label used. Good results have been obtained with both HPLC and silica gel chromatography

Protocol for Labeling SNAP-tag Fusion Proteins with BG-substrates

Once the amine-containing precursor has been converted into a BG-substrate it can be used to label SNAP-tag fusion proteins. SNAP-tag fusions can be labeled in cell lysates or as purified protein. For expression of fusion proteins with the SNAP-tag, refer to instructions supplied with the SNAP-tag plasmids.

We recommend using a 1.5 fold excess of substrate over SNAP-tag fusion protein and incubating for 1 hour at room temperature. Typical concentrations are 30 µM substrate and 20 µM SNAP-tag fusion protein. The labeling incubation is followed by a separation step such as dialysis or spin column separation to remove unreacted substrate.

Notes

Unlabeled protein samples should be stored at –20°C, or at –80°C for long-term storage. Many proteins benefit from the addition of glycerol for frozen storage, typically 20% v/v. Handling at temperatures above 0°C should be minimized by thawing the unlabeled protein samples shortly before use, and keeping them on ice until just before the labeling reaction.

We recommend the routine addition of 1 mM DTT to all buffers used for handling, labeling and storage of SNAP-tag fusion proteins. The stability of the SNAP-tag is improved in the presence of reducing agents; however it can also be labeled in their absence.

If a particular fusion protein requires buffers without reducing agents, pay particular attention to minimize all handling steps of the protein above 4°C prior to the labeling reaction.

The SNAP-tag labeling reaction works well between pH 5.0 and pH 10.0. The salt concentration may need to be optimized for your particular fusion protein, although the SNAP-tag labeling reaction has been shown to work at a broad range of ionic strengths (NaCl concentrations between 15 mM and 1 M). If the buffer conditions used for labeling lead to insolubility of your protein, we recommend testing a range of pH and ionic strengths. For a range of fusion proteins we have found ionic strengths for monovalent salts (e.g. sodium chloride) from 50 mM to 250 mM helpful.

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.Ionic detergents (e.g. SDS) should be avoided.

The maximum degree of labeling possible with the SNAP-tag is one molecule of label per molecule of fusion protein.

Storage and Handling of Labeled SNAP-tag Fusion Proteins

Labeled SNAP-tag fusion proteins should be stored at concentrations of 1 mg/ml or less at 4°C protected from light. Sodium azide may be added to 2 mM final concentration. Under these conditions the labeled protein should be stable at 2–6°C for several months. If freezing is required, it may be best to shock freeze by rapidly cooling the sample to a temperature significantly below 0°C in the presence of 20% (v/v) glycerol.

Labeled protein is linked to the SNAP-tag label by a covalent bond. Therefore the conjugates are highly stable. DTT or other reducing agent is not required in the storage buffers. As the fusion protein is covalently labeled, labeled protein may be detected after harsh or even denaturing treatments, such as chromatographic or electrophoretic separation, including SDS-PAGE.

Note: Avoid repeated freezing and thawing. Protect from light if the protein is labeled with a fluorophore.

Troubleshooting

Labeling reaction

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 double the ratio of label to protein in the labeling reaction. Both approaches may be combined. If you still have poor labeling results, we recommend checking the activity of the SNAP-tag.

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.

Inefficient Labeling

If the degree of labeling is extremely low, the labeling reaction was probably inefficient. A number of reasons can lead to such inefficient labeling:

If you added the substrate dissolved in DMSO at greater than 1% of the volume of protein solution, the higher DMSO concentration may slow down the reaction rate. At lower substrate concentrations the labeling time should be increased to 2 hours.

The activity of the SNAP-tag may have been partially or completely lost. This may be due to extended storage of non-reacted SNAP-tag fusion proteins at 4°C or greater. The sensitivity of the SNAP-tag to inactivation is also significantly increased if no reducing agent is added (such as DTT at 1 mM).

(see other side)

CERTIFICATE OF ANALYSIS

If you believe that the activity of the SNAP-tag is affected, we recommend analyzing a small fraction of it on an SDS-PAGE gel using SNAP-Vista® Green (NEB #S9147) to confirm that the SNAP-tag is active.

If you encounter problems with the activity we recommend thawing another sample of your protein or reexpressing and repurifying the SNAP-tag fusion protein following the advice given in the $pSNAP_f$ Vector (NEB #9183) instructions.

Reference

1. Howland, S.W. et al. (2008) *J. Immunother.*, 31, 607-619.