

BG-PEG-NH₂



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S9150S 004120815081

S9150S

2 mg Lot: 0041208

Store at: -20°C Exp: 8/15

Introduction

BG-PEG-NH₂ is an amine-terminated building block for the one-step synthesis of SNAP-tag® substrates from NHS esters or other activated carboxylic esters on labels or surfaces. This building block allows you to make custom SNAP-tag substrates for labeling SNAP-tag fusion proteins for a wide range of applications. BG-PEG-NH₂ is particularly useful for the synthesis of SNAP-tag substrates with affinity ligands or solid surfaces.

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 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 fusion proteins with the SNAP-tag substrate is described in this document.

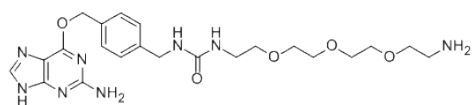


Figure 1. Structure of BG-PEG-NH₂ (MW 488.5 g/mol)

Storage

Dry BG-PEG-NH₂ should be stored at -20°C.

BG-PEG-NH₂ is soluble in anhydrous N,N-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Choose a solvent volume that will be compatible with the final use. Heating at 37°C, vortexing and/or sonication will aid dissolution.

Unused BG-PEG-NH₂ substrate dissolved in DMF or DMSO should be stored at -20°C.

Quality Control

Purity and Characterization: Purity of BG-PEG-NH₂ was determined to be 95% by HPLC analysis. Molecular weight [M+H]⁺ was determined by MS to be 489.3 (489.3 expected)

Reaction Conditions for Chemical Coupling

We recommend that coupling reactions between BG-PEG-NH₂ and NHS esters or activated carboxylic acids take place in anhydrous N,N-dimethylformamide (DMF) using triethylamine as a base.

Typical reactions use approximately equimolar quantities of the BG-PEG-NH₂ and the desired NHS-label at 5–20 mM final concentration in the presence of a 1.5 fold molar excess of triethylamine in DMF. The reaction is generally performed at 30°C overnight.

Example Reaction: Coupling of BG-PEG-NH₂ to (+)-biotin-N-hydroxysuccinimide ester:

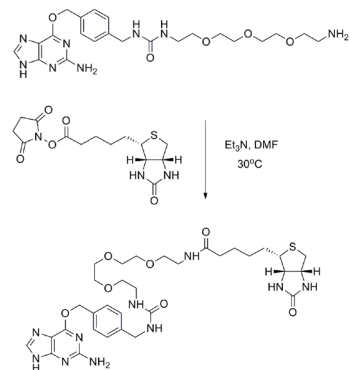


Figure 2. Coupling of BG-PEG-NH₂ to (+)-biotin-N-hydroxysuccinimide ester. Triethylamine (0.9 µl, 6.2 µmol, 1.5 eq.) was added to a solution of BG-PEG-NH₂ (2.0 mg, 4.1 µmol) in 200 µl DMF in an Eppendorf tube. A solution of (+)-biotin-N-hydroxysuccinimide ester (1.4 mg, 4.1 µmol, 1.0 eq.) in 200 µl DMF was added to the mixture, which was shaken at 30°C overnight. The solvent was evaporated under vacuum. The redissolved solid was adsorbed on silica gel and purified by flash column chromatography (CH₂Cl₂/MeOH 5:1) to give a colorless viscous solid after solvent evaporation.

The purification strategy will depend on the label used and the reaction scale. Good results have been obtained with both HPLC and silica gel chromatography.

Use with Biacore® CM5 Chips

Surfaces such as Biacore® CM5 chips have been modified with BG-PEG-NH₂ so that they covalently immobilize SNAP-tag fusion proteins. Surface activation is performed using standard amino-coupling procedures (exposure to NHS and EDC). Refer to Biacore® CM5 chips manufacturer's protocol for detailed instruction.

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

Once the building block has been converted into a BG-substrate it can be used to label SNAP-tag fusion proteins. These can be labeled in cell lysates or as purified protein.

For labeling, we recommend using a 1.5 fold excess of substrate to SNAP-tag fusion protein and incubation for 1 hour at room temperature. Typical concentrations are 30 µM substrate and 20 µM SNAP-tag fusion protein. The labeling incubation can be followed by a separation step such as dialysis or spin column separation to remove unreacted substrate, if desired.

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, 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. Sodium azide may be added to 2 mM final concentration to prevent bacterial growth. 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 agents are not required in the storage buffers. Since 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.

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: Double 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.

(see other side)

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

DMSO at greater than 1% of the volume of protein solution may slow the reaction rate. Lower substrate concentrations also slow the reaction rate. Increasing the reaction time may be helpful in these cases.

Activity of the SNAP-tag can sometimes be partially or completely lost. This can be caused by 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

If you believe that the activity of the SNAP-tag is affected, we recommend analyzing a small fraction of it on a SDS-PAGE gel using the 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 re-expressing and repurifying the SNAP-tag fusion protein following the advice given in the SNAP-tag plasmid instructions.

References

1. Kindermann, M. et al. (2003) *JACS* 125, 7810–7811.
2. Sielaff, I. et al. (2006) *ChemBiochem* 7, 194–202.
3. Kufer, S.K. et al. (2005) *Eur. Biophys. J.* 35, 72–78.
4. Huber, W. et al. (2004) *Anal. Biochem.* 333, 280–288.

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