

# BC-PEG-NH<sub>2</sub>



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S9222S 001130115011

## S9222S

**2 mg**                      **Lot: 0011301**

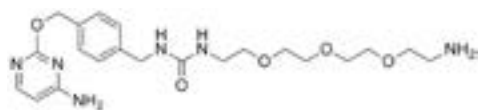
**Store at: -20°C**                      **Exp: 1/15**

### Introduction

BC-PEG-NH<sub>2</sub> is an amine-terminated building block for the one-step synthesis of CLIP-tag substrates from NHS esters or other activated carboxylic esters on labels or surfaces. This building block allows you to make custom CLIP-tag substrates for labeling CLIP-tag fusion proteins for a wide range of applications. BC-PEG-NH<sub>2</sub> is particularly useful for the synthesis of CLIP-tag substrates with affinity ligands or solid surfaces.

The CLIP-tag protein labeling system enables the specific, covalent attachment of virtually any molecule to a protein of interest. CLIP-tag is a protein tag based on human O<sup>6</sup>-alkylguanine-DNA-alkyltransferase (hAGT). CLIP-tag substrates are derivatives of benzylcytosine (BC). In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the reactive cysteine of CLIP-tag forming a stable thioether link. Although CLIP-tag is based on the same protein as SNAP-tag<sup>®</sup>, the benzylcytosine substrates form a separate class of substrates, different from the benzylguanine substrates recognized by SNAP-tag. CLIP-tag and SNAP-tag can be used for orthogonal and complementary labeling of two proteins simultaneously in the same cells.

There are two steps to using this system: sub-cloning and expression of the protein of interest as a CLIP-tag fusion, and labeling of the fusion with the CLIP-tag substrate of choice. Expression of CLIP-tag fusion proteins is described in the documentation supplied with CLIP-tag plasmids. The labeling of fusion proteins with the CLIP-tag substrate is described in this document.



**Figure 1.** Structure of BC-PEG-NH<sub>2</sub> (MW 448.5 g/mol)

### Storage

Dry BC-PEG-NH<sub>2</sub> should be stored at -20°C.

BC-PEG-NH<sub>2</sub> 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 BC-PEG-NH<sub>2</sub> substrate dissolved in DMF or DMSO should be stored at -20°C.

### Quality Controls

**Purity and Characterization:** Purity of BC-PEG-NH<sub>2</sub> was determined by HPLC analysis to be 99%. Molecular weight [M+H]<sup>+</sup> was determined by MS to be 449.2 (449.3 expected).

### Reaction Conditions for Chemical Coupling

We recommend that coupling reactions between BC-PEG-NH<sub>2</sub> and NHS esters or activated carboxylic acids take place in anhydrous N, N-dimethylformamide (DMF) by using triethylamine as a base.

Typical reactions use approximately equimolar quantities of the BC-PEG-NH<sub>2</sub> 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. It is convenient to use similar volumes of the BC-PEG-NH<sub>2</sub> and the NHS-label. The reaction is generally performed at 30°C overnight.

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.

### Protocol for Labeling CLIP-tag Fusion Proteins with BC-substrates

Once the building block has been converted into a BC-substrate it can be used to label CLIP-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 CLIP-tag fusion protein and incubation for 1 hour at room temperature. Typical concentrations are 30 μM substrate and 20 μM CLIP-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 CLIP-tag fusion proteins. The stability of the CLIP-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 CLIP-tag labeling reaction works well between pH 5.0 and pH 10.0. The salt concentration may need to be optimized for the particular fusion protein, although the CLIP-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 the 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 CLIP-tag. Ionic detergents (e.g. SDS) should be avoided.

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

### Storage and Handling of Labeled CLIP-tag Fusion Proteins

Labeled CLIP-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 CLIP-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 poor labeling continues, we recommend checking the activity of the CLIP-tag.

If the fusion protein is particularly sensitive to degradation or to loss of activity, try reducing the labeling time or decreasing the labeling temperature. We recommend overnight incubation when labeling at 4°C.

#### 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 CLIP-tag can sometimes be partially or completely lost. This can be caused by extended storage of non-reacted CLIP-tag fusion proteins at 4°C or greater. The sensitivity of the CLIP-tag to inactivation is also significantly increased if no reducing agent is added.

If it is believed that the activity of the CLIP-tag is affected, we recommend analyzing a small fraction of it on an SDS-PAGE gel using CLIP-Vista Green (NEB #S9235) to confirm that the CLIP-tag is active.

If problems with the activity is experienced, we recommend thawing another sample of the protein or re-expressing and repurifying the CLIP-tag fusion protein following the advice given in the CLIP-tag plasmid instructions.

#### Reference

1. Gautier, A. et al. (2008) *Chemistry & Biology* 15, 128–136.

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