Click Chemistry Tools
Bioconjugate Technology Company

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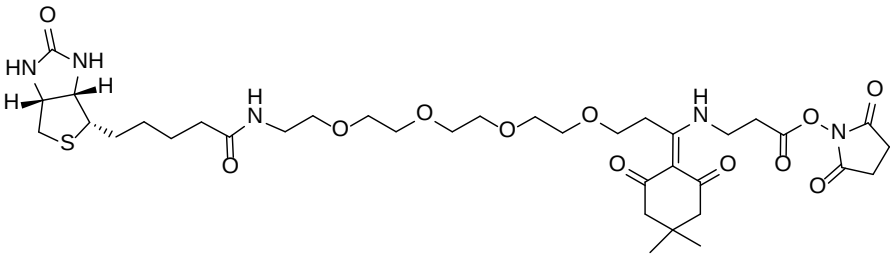
Dde Biotin-PEG4-NHS ester

Product No.:	1207
Product Name:	Dde Biotin-PEG4-NHS ester
Chemical Structure:	
Molecular Weight:	781.91
Chemical Composition:	$C_{36}H_{55}N_5O_{12}S$
Solubility:	DMSO, DMF, THF, DCM
Storage:	Upon receipt store at -20°C. Product shipped at ambient temperature

Introduction

Dde Biotin-PEG4-NHS ester is amine-reactive, hydrazine cleavable biotinylation reagents. Biotinylated protein targets can be selectively captured through conventional affinity biotin - streptavidin purification, and released under mild buffer conditions with a 2% hydrazine solution for subsequent identification by mass spectrometry, ELISA, dot blot, or Western blot applications.

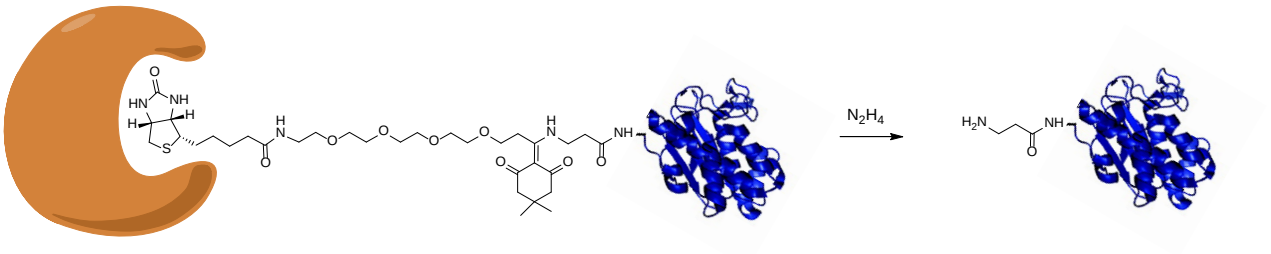


Figure 1. Schematic representation of dde biotin probe cleavage.

Protein Requirements

- The protein to be biotinylated must be highly purified and its molecular weight known (e.g. 20-200 kDa).
- The protein must have available primary amines (e.g. N-terminus or lysine amino acid residues).
- Proteins must be free of exogenous primary amines (e.g. glycine or Tris) prior to labeling with NHS esters, if present these compounds must be removed with desalting spin columns.

Important Considerations

- After reconstitution Dde Biotin-PEG4-NHS reagent in DMSO, use it immediately. NHS-esters readily hydrolyze and become non-reactive. Use only freshly prepared reagent and discard any unused reconstituted reagent.
- For maximum reproducibility, it is preferable to buffer exchange proteins into BupH Buffer (pH 7.5) with the spin columns provided prior to biotinylation. This simple procedure guarantees maximum consistency during the labeling reaction.

Protein Preparation

1. If the lyophilized protein (50-500 µg) to be biotinylated is pure and free of exogenous amines, resuspend in 100 µL BupH buffer (pH 7.5) to obtain a 0.5-5 mg/mL solution. Proceed to Biotin Labeling Reaction.
2. If the lyophilized protein is known to contain exogenous amines (e.g. Tris, glycine) resuspend in 100 µL BupH buffer (pH 7.5) then proceed with buffer exchange using spin columns.
3. If the protein to be biotinylated (50-500 µg) is already in 100 µL buffer solution (e.g. 0.5-5 mg/mL PBS), proceed to buffer exchange into BupH buffer (pH 7.5) with spin columns.

Biotin Labeling Reaction

1. Select desired excess Dde Biotin-PEG4-NHS to use during the labeling reaction (e.g. 10-fold excess).
2. Immediately before use prepare a 10 mM stock solution of Dde Biotin-PEG4-NHS ester by dissolving reagent in anhydrous DMSO. Vortex for 1-2 minutes to mix well.
   To make 10 mM stock solution add 128 µL of DMSO to 1 mg vial or 640 µL of DMSO to 5 mg.
3. Add required volume of stock solution of Dde Biotin-PEG4-NHS ester to protein solution, pipette the mixture up and down several times to mix.
4. Allow labeling reaction to proceed for 1 hour at room temperature.
5. Remove excess of Dde Biotin-PEG4-NHS reagent with an appropriate desalting column.

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Procedure for Capture of Biotin Labeled Proteins

The capture protocol provided is optimized for our high capacity streptavidin agarose (Click Chemistry Tools, cat# 1051). The typical range for biotin labeled protein is 20-200 µg of total protein per 100 µL of settled resin. This can be scaled up or down, depending on the amount of biotin labeled protein to be captured on the resin.

1. Thoroughly mix and vortex the high capacity streptavidin agarose resin to resuspend. Transfer the desired volume of resin to a 1.7 mL microfuge tube. Use a P200 pipette with a cut tip to ensure that the resin is transferred properly.
2. Centrifuge the resin at 500 x g for 2 min to pellet the resin.
3. Carefully remove and discard the storage buffer (contains Kathon as preservative).
4. Resuspend the pellet in an equal volume of PBS pH 7.0 (50% slurry in PBS). Vortex to mix thoroughly. Centrifuge at 500 x g for 2 min. Carefully remove and discard the wash buffer. Repeat this wash step 2 additional times.
5. After equilibration into PBS pH 7.0, add required volume of biotin labeled proteins (see above). The volume ratio of settled resin:labeled protein should not exceed 2:1 (for example, if using 100 µL of settled resin, do not use less than 50 µL of protein solution). After addition of biotin labeled proteins, vortex briefly and incubate for 30 min at room temperature with agitation.
6. After incubation, centrifuge at 500 x g for 2 min. Retain the supernatant (flow through) for analysis by UV/VIS and/or SDS-PAGE.
7. Wash the resin by the addition of PBS pH 7.0 (use a volume equal to the volume of resin). Vortex briefly to resuspend the pellet. Centrifuge at 500 x g for 2 min. Carefully remove and discard the supernatant. Repeat this wash step 3-6 times.
8. **Note:** Do not use buffers with pH>7.8 or those that contain PBS as this can cause cleavage.
9. The resin now contains bound biotin labeled protein and is ready for cleavage/elution.

Procedure for Cleavage

1. Resuspend the washed resin in elution buffer (100mM sodium phosphate + 2%(v/v) hydrazine)
2. Incubate at room temperature for 30-120 min with agitation.
3. Collect the eluant by centrifugation or using an empty spin column.
4. Wash the resin 2 additional times with PBS. Retain for analysis.

Selected Publications

1. Yang Y., *et al.* (2013). Cleavable Trifunctional Biotin Reagents for Protein Labeling, Capture, and Release. *Chem. Commun.*, **49:**5366-06.

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