

EZ-Link HPDP-Biotin

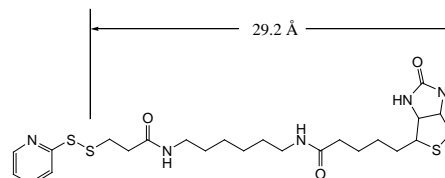
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21341

Number	Description
21341	EZ-Link HPDP-Biotin , N-[6-(Biotinamido)hexyl]-3'-(2'-pyridyldithio)propionamide, 50mg Formula: C ₂₄ H ₃₇ N ₅ O ₃ S ₃ Molecular Weight: 539.78 Spacer Arm Length: 29.2Å



Storage: Upon receipt store product at 4°C. Product shipped at ambient temperature.

Introduction

Thermo Scientific™ EZ-Link™ HPDP-Biotin is a membrane-permeable biotin labeling reagent that reacts with sulfhydryl (-SH) groups. The resulting disulfide bond between the target sulfhydryl molecule and the biotin group can be cleaved by reducing agents to release the biotin group and regenerate the protein (or peptide) in its original, unmodified form (Figure 1). Labeling with HPDP-Biotin is convenient when using immobilized avidin, streptavidin or Thermo Scientific™ NeutrAvidin™ Protein to purify the target molecules for reducing SDS-PAGE or mass analysis; the captured biotinylated molecules can be efficiently eluted from the support by cleaving the disulfide bond with dithiothreitol (DTT) or other reducing agent rather than by attempting to dissociate the high affinity interaction between avidin and biotin with strong acid or denaturant.

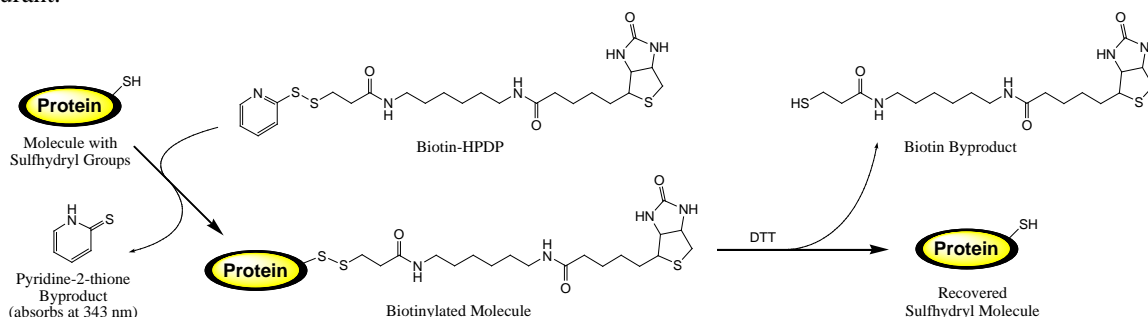


Figure 1. Reaction scheme for biotinylation of sulfhydryl molecules with HPDP-Biotin .

Important Product Information

- The 2-pyridyldithio group of HPDP-Biotin reacts optimally with free (reduced) sulfhydryls at pH 7-8. Reaction buffers must be free of thiols and disulfide reducing agents until quenching or reduction of the 2-pyridyldithiol is desired.
- The reaction of HPDP-Biotin to sulfhydryl groups results in displacement of a pyridine-2-thione group, the concentration of which may be determined by measuring the absorbance at 343nm (see Additional Information section). For reactions of sufficient concentration, this measurement allows reaction progress to be monitored.
- To make sulfhydryl groups (-SH) available for labeling, reduce peptide disulfide bonds with Thermo Scientific Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). Reduce protein disulfide bonds using 5-10mM DTT or TCEP solution (Product No. 77720), followed by desalting. Be aware that proteins (e.g., antibodies) may be inactivated by complete reduction of their disulfide bonds. Sulfhydryls can be added to primary amine sites using SATA (Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101).
- When biotinylating proteins in solution, excess nonreacted biotin is easily removed by size exclusion using either desalting columns or dialysis (see Related Thermo Scientific Products). Depending on the downstream application, biotinylated peptides may be purified from excess nonreacted biotin reagent using C18 resin.

Example Procedure for Protein Labeling with HPDP-Biotin

A. Additional Materials Required

- Reaction Buffer: Sulfhydryl-free buffer such as phosphate-buffered saline (PBS, Product No. 28374). Including 1mM EDTA in the buffer helps maintain reduced sulfhydryls until they have the opportunity to react with the HPDP-Biotin.
- Solvent: HPDP-Biotin is not soluble in aqueous buffer; it must be dissolved in organic solvent before addition to an aqueous reaction. Use dimethylsulfoxide (DMSO, Product No. 20688) or dimethylformamide (DMF, No. 20672).
- (Optional): For separating labeled protein from excess nonreacted HPDP-Biotin: Thermo Scientific™ Zeba™ Spin Desalting Columns (e.g., Product No. 89891) or Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassettes (e.g., Product No. 66382).

B. Material Preparation

HPDP-Biotin Stock Solution: Prepare 4mM HPDP-Biotin stock solution by adding 2.2mg HPDP-Biotin to 1.0mL of Solvent (e.g., DMF). To ensure complete dissolution of the reagent, gently warm the mixture to 37°C and vortex or sonicate. This stock can be aliquoted and stored frozen.

C. Biotinylation of β-D-galactosidase (Protein)

1. Dissolve 2mg of reduced β-D-galactosidase in 1mL Reaction Buffer.
2. Add 100μL of HPDP-Biotin Stock Solution to 1mL of protein solution (results in 0.4mM Biotin HPDP).
3. Vortex to mix and then incubate reaction mixture for 2 hours at room temperature.
4. Desalt the reaction mixture using a Desalting Column equilibrated with Reaction Buffer or other suitable storage buffer.

Additional Information

A. Pyridine-2-Thione Assay to Monitor Reaction

1. Immediately before (and/or after) adding HPDP-Biotin to the protein sample, measure and record the absorbance at 343nm of the protein sample compared to a buffer (e.g., PBS) blank.
2. At various time-points after beginning the labeling reaction, measure and record the absorbance at 343nm of the sample.
3. Calculate the change in absorbance: $\Delta A_{343} = (\text{Ave. } A_{343} \text{ at time-point}) - (\text{Ave. } A_{343} \text{ at time 0})$
4. Calculate the molar ratio of biotin to protein using the following equation:

$$\frac{\Delta A}{8080} \times \frac{\text{MW of Protein}}{\text{mg/ml of Protein}} = \text{moles of HPDP-Biotin reaction (biotinylation) per mole of Protein}$$

Where the value 8080 reflects the extinction coefficient for pyridine-2-thione at 343nm: $8.08 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$.

B. Determination of Biotin Incorporation

Biotin incorporation can be estimated using the HABA (4'-hydroxyazobenzene-2-carboxylic acid) method. The Thermo Scientific™ Pierce™ Biotin Quantitation Kit (Product No. 28005) contains a premix of HABA, avidin and a biotinylated protein control supplied in convenient No-Weigh™ Microtube packaging.

Related Thermo Scientific Products

26101	Traut's Reagent, 500mg
26102	SATA (<i>N</i> -succinimidyl <i>S</i> -acetylthioacetate), 50mg
20291	No-Weigh Dithiothreitol (DTT), 48 × 7.7mg microtubes
20408	2-Mercaptoethylamine•HCl, 6 × 6mg
20490	TCEP•HCl, 1g
28372	BupH Phosphate Buffered Saline Packs, 40 packs
69576	Slide-A-Lyzer MINI Dialysis Unit Kit, for 10-100µL sample volumes, 10 units plus float
66382	Slide-A-Lyzer Dialysis Cassette Kits, 10K MWCO, for 0.5-3mL samples
89891	Zeba Spin Desalting Columns, 7K MWCO, 5mL, 5/pkg
20347	Streptavidin Agarose Resin, 2mL
29200	NeutrAvidin Agarose Resin, 5mL
28005	EZ Biotin Quantitation Kit
46610	Fluorescence Biotin Quantitation Kit
21126	Streptavidin, Horseradish Peroxidase Conjugated, 1mg

Product References

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