INSTRUCTIONS

# EZ-Link HPDP-Biotin, No-Weigh Format

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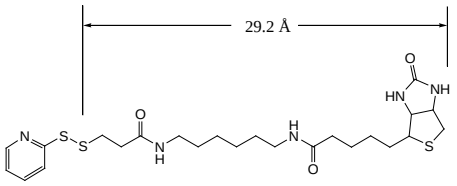
A35390

| Number | Description |
|---|---|
| **A35390** | **EZ-Link HPDP-Biotin, No-Weigh Format** N-[6-(Biotinamido)hexyl]-3´-(2´-pyridyldithio)propionamide, 10 × 1mg |

Formula: $C_{24}H_{37}N_5O_3S_3$

Molecular Weight: 539.78

Spacer Arm Length: 29.2Å




**Storage:** Upon receipt store product at 4°C. Product shipped on ice.

**For Research Use Only. Not for use in diagnostic procedures.**

## Introduction

Thermo Scientific™ EZ-Link™ HPDP-Biotin, No-weigh format 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.

Thermo Scientific™ No-Weigh™ products are specialty reagents provided in a pre-aliquoted format. The pre-weighed packaging prevents the loss of reagent reactivity and contamination over time by eliminating the repetitive opening and closing of the vial. The format enables use of a fresh vial of reagent each time, eliminating the hassle of weighing small amounts of reagents and reducing concerns over reagent stability.

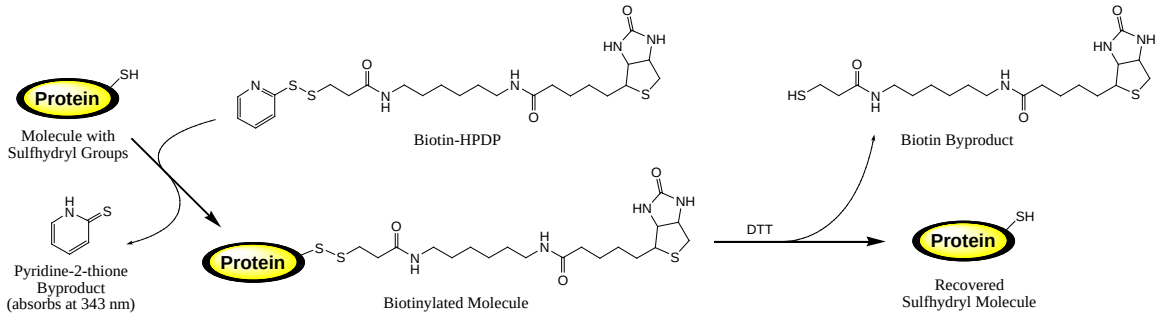



**Figure 1.** Reaction scheme for biotinylation of sulfhydryl molecules with HPDP-Biotin .

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## 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). Gentle heating to around 55-60°C may be required for complete dissolution.
- (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 463µL of solvent (DMF or DMSO) to 1 vial (1mg) of HPDP-Biotin. To ensure complete dissolution of the reagent, replace the cap and gently warm the mixture to 50-60°C and vortex or sonicate. The maximum useable volume of each tube is 800µL.

### 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: $\Delta A_{343}$ = (Ave. $A_{343}$ at time-point) – (Ave. $A_{343}$ 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: $8.08 \times 10^3\ M^{-1}cm^{-1}$.

### 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

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|---|---|
| **26101** | **Traut's Reagent,** 500mg |
| **26102** | **SATA (*N*-succinimidyl S-acetylthioacetate),** 50mg |
| **20291** | **No-Weigh Dithiothreitol (DTT),** 48 × 7.7mg microtubes |
| **20408** | **2-Mercaptoethylamine•HCl,** 6 × 6mg |
| **20490** | **TCEP•HCl,** 1g |
| **A35349** | **Pierce TCEP-HCl, No-Weigh™ format, 10 x 1mg** |
| **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 |

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## Product References

Demidov, V.V., *et al*. (2006). Fast complementation of split fluorescent protein triggered by DNA hybridization. *Proc Natl Acad Sci USA* **103:**2052-6.

Greco, T.M., *et al*. (2006). Identification of S-nitrosylation motifs by site-specific mapping of the S-nitrosocysteine proteome in human vascular smooth muscles cells. *Proc Natl Acad Sci USA* **103:**7420-5.

Hermanson, G.T. (1996). *Bioconjugate Techniques*. Academic Press, Inc.

Keinker, P.K., *et al*. (2003). Sizing the protein translocation pathway of colicin Ia channels. *J Gen Physiol* **122:**161-76.

Le Moan, N., *et al*. (2006). The *Saccharomyces cerevisiae* proteome of oxidized protein thiols: contrasted functions for the thioredoxin and glutathione pathways. *J Biol Chem* **281:**10420-30.

Politis, E.G., *et al*. (2005). Transmembrane topology of the protein palmitoyl transferase Akr1. *J Biol Chem* **280:** 10156-63.

Sugawara, E., *et al*. (2006). *Pseudomonas aeruginosa* porin OprF exists in two different conformations. *J Biol Chem* **281:**16220-9.

Tolia, A., *et al*. (2006). Contribution of presenilin transmembrane domains 6 and 7 to a water-containing cavity in the γ-secretase complex. *J Biol Chem* **281:**27633-42.