

# EZ-Link<sup>®</sup> PFP-Biotin

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## Number

21218

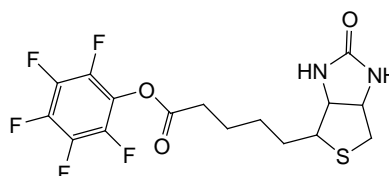
## Description

**EZ-Link PFP-Biotin, 50mg**

Molecular Weight: 410.36

Spacer Arm: 9.6Å

Maximum Solubility: 25mg/mL in DMF



**Storage:** Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.

## Introduction

The Thermo Scientific EZ-Link PFP-Biotin is a biotin labeling reagent that reacts with primary and secondary amines. The pentafluorophenyl (PFP) ester-activated biotin is less subject to hydrolysis than NHS esters, resulting in more efficient reactions. EZ-Link PFP-Biotin must be first dissolved in a minimal amount of an organic solvent, such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF) and then added to the buffer containing the protein or other molecule. The reagent forms an emulsion that allows the reaction to proceed.

## Important Product Information

- EZ-Link PFP-Biotin is moisture-sensitive. Store the vial of biotin reagent at 4°C with desiccant. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening.
- As directed in the procedure, dissolve the biotin reagent immediately before use. The PFP moiety readily hydrolyzes and becomes non-reactive; therefore, weigh and dissolve only a small amount of the reagent at a time, and do not prepare stock solutions for storage. Discard any unused reconstituted reagent.
- Avoid buffers containing primary amines (e.g., Tris or glycine) as these will compete with the reaction. If necessary, dialyze or desalt to exchange the protein sample into an amine-free buffer such as phosphate-buffered saline (PBS).
- When biotinylating proteins in solution, excess nonreacted biotin is easily removed by size exclusion using either desalting columns or dialysis (see Additional Information and Related Thermo Scientific Products). A 10mL desalting column is best suited for processing biotinylation reactions involving 1-10mg of protein in approximately 0.5-2mL. For smaller amounts of protein and/or smaller reaction volumes, both the biotinylation reaction and subsequent buffer exchange may be performed in a single Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit. For larger reaction volumes than can be processed with a desalting column, either split the sample between two columns or use an appropriate Slide-A-Lyzer<sup>®</sup> Dialysis Cassette.

## General Procedure for Biotinyating IgG and other Proteins

The following protocol typically results in approximately two biotin molecules per IgG. The degree of EZ-Link PFP-Biotin incorporation can vary depending on the parameters of the biotinylation reaction, including protein concentration, EZ-Link PFP-Biotin concentration, pH and time. Commonly used reaction conditions include incubation at 4-37°C, pH values from 7 to 9, and incubation times from a few minutes to overnight.

1. Dissolve 2mg of IgG in 1mL of PBS (for example, 0.1M sodium phosphate 0.15M NaCl, pH 7.2).
2. Immediately before use, dissolve 1mg of EZ-Link PFP-Biotin in 75µL of DMF or DMSO. Add 25µL of the PFP-Biotin solution to the IgG solution.
3. Incubate the reaction on ice for two hours or at room temperature for 30 minutes.
4. Remove nonreacted biotin by dialysis or gel filtration.
5. Store the biotinylated protein at 4°C until ready for use.

## Additional Information

### A. Determination of Biotin Incorporation

Biotin incorporation can be estimated using the HABA (4'-hydroxyazobenzene-2-carboxylic acid) method. In solution, the HABA dye binds avidin, forming a complex with maximal absorption at 500nm. When biotin is added to the solution, its higher affinity for avidin displaces the HABA and the absorption at 500nm decreases proportionately. The absorbance of the HABA-avidin solution is measured before and after adding the biotin-containing sample. The change in absorbance relates to the amount of biotin in the sample. The Thermo Scientific Pierce Biotin Quantitation Kit (Product No. 28005) contains a biotinylated HRP control and a premix of HABA and avidin supplied in convenient No-Weigh™ Microtube packaging, which eliminates difficulties associated with weighing small quantities of reagent.

### B. Please visit the website for additional information related to this product including the following:

- Tech Tip #14: Perform labeling and other reactions in Slide-A-Lyzer Dialysis Cassettes
- HABA Calculator

## Related Thermo Scientific Products

28372	<b>BupH™ Phosphate Buffered Saline Packs, 40 packs</b>
69576	<b>Slide-A-Lyzer MINI Dialysis Unit Kit, 10K MWCO, 0.1mL</b>
66382, 66807	<b>Slide-A-Lyzer Dialysis Cassette Kits, 10K MWCO, 3mL and 12mL, respectively</b>
43233	<b>Dextran Desalting Columns, 5K MWCO, 10mL, 5/pkg</b>
20351	<b>Streptavidin Agarose Columns, 1mL, 5/pkg</b>
20219	<b>Pierce Avidin Agarose, 5mL</b>
20347	<b>Streptavidin Agarose Resin, 2mL</b>
29200	<b>NeutrAvidin® Agarose Resin, 5mL</b>
28005	<b>Pierce Biotin Quantitation Kit</b>
21126	<b>Streptavidin, Horseradish Peroxidase Conjugated, 1mg</b>

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

**No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).**

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at [www.thermoscientific.com/pierce](http://www.thermoscientific.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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