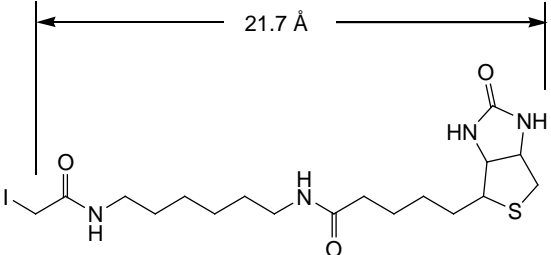
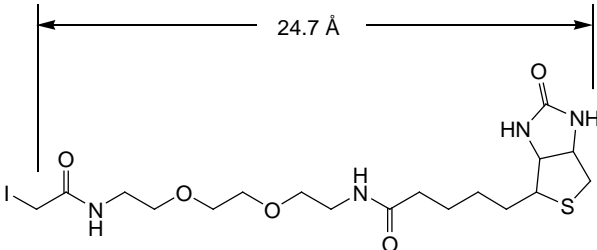


EZ-Link[®] Iodoacetyl-LC-Biotin

EZ-Link Iodoacetyl-PEG₂-Biotin

21333 21334

0254.5

Number	Description
21333	<p>EZ-Link Iodoacetyl-LC-Biotin (N-Iodoacetyl-N-biotinylhexylenediamine), 50mg</p> <p>Formula: C₁₈H₃₁IN₄O₃S</p> <p>Molecular Weight: 510.43</p> <p>Spacer Arm: 27.1Å</p> <p>Net Mass Addition: 382.53</p> <p>Solubility: > 2mg/mL in DMF for subsequent dilution in water or buffer</p> 
21334	<p>EZ-Link Iodoacetyl-PEG₂-Biotin (+)-Biotinyl-iodoacetamidyl-3,6-dioxaoctanediamine), 50mg</p> <p>Formula: C₁₈H₃₁IN₄O₅S</p> <p>Molecular Weight: 542.43</p> <p>Spacer Arm: 24.7Å</p> <p>Net Mass Addition: 414.19</p> <p>Solubility: ≥ 25mg/mL in water or buffer</p> 

Storage: Upon receipt store product at 4°C protected from light and moisture. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific EZ-Link Iodoacetyl-LC-Biotin and Iodoacetyl-PEG₂-Biotin are long-chain, sulfhydryl-reactive biotinylation reagents. Iodoacetyl-LC-Biotin is not soluble in water and must be dissolved in an organic solvent such as DMF before further dilution in aqueous solutions. Iodoacetyl-PEG₂-Biotin has a hydrophilic polyethylene glycol (PEG) spacer arm that imparts high water-solubility to the reagent and confers added water-solubility to modified molecules.

For both reagents, reaction of the iodoacetyl group with a sulfhydryl (-SH) group is rapid and specific, especially when only a slight reagent-to-sulfhydryl molar excess is used and the reaction is performed at pH 8.3 (7.5-8.5).¹⁻³ The reaction occurs by nucleophilic substitution of iodine with a thiol (sulfhydryl) group, resulting in a stable thioether bond (Figure 1). If thiols are unavailable on the molecule, reaction can occur with histidyl side chains at pH 6.9-7.0, but the reaction must be allowed to proceed for at least one week. Iodoacetyl reagents also will react with amino groups at pH > 10.

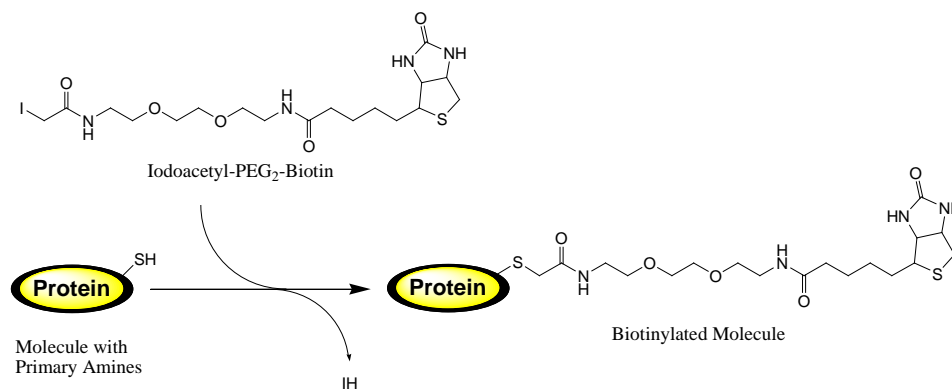


Figure 1. Biotinylation through thioether bond formation using EZ-Link Iodoacetyl-PEG₂-Biotin.

Important Product Information

- Iodoacetyl Biotin Reagents are moisture-sensitive. To avoid moisture condensation in the container, equilibrate vial to room temperature before opening. Store Iodoacetyl Biotin Reagent protected from light at 4-8°C and desiccated.
- Perform reactions in buffers that are free of thiols (sulfhydryl groups).
- Molecules to be reacted with the iodoacetyl moiety must have free (reduced) sulfhydryls. Reduce peptide disulfide bonds with Thermo Scientific Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). Reduce disulfide bonds in high molecular weight proteins using 5mM TCEP (1:100 dilution of Thermo Scientific Bond-Breaker TCEP Solution, Product No. 77720) for 30 minutes at room temperature, followed by TCEP removal using a desalting column (e.g., Thermo Scientific Zeba Spin Desalting Columns). Proteins (e.g., antibodies) can be inactivated by complete reduction of their disulfide bonds. Selective reduction of hinge-region disulfide bonds in IgG can be accomplished with 2-Mercaptoethylamine•HCl (2-MEA, Product No. 20408). Sulfhydryls can be added to molecules using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102 or SAT(PEG)₄, Product No. 26099) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101), which modify primary amines.

Reduction of IgG and Biotinylation with Iodoacetyl Biotin Reagent

The following method uses 2-mercaptoethylamine•HCl (2-MEA) as a selective and mild disulfide-cleaving reagent for reducing whole IgG in preparation for biotinylation (see Important Product Information).⁴ The protocol can be modified for other proteins, peptides and other molecules. The protein concentration during the mild reduction is not as critical as the absolute concentration of 2-MEA, which is 50mM; 1-10mg IgG/mL can be effectively reduced at this 2-MEA concentration. Generally, a 3- to 5-fold molar excess of iodoacetyl biotin reagent to sulfhydryl groups is sufficient to obtain efficient modification. Specific applications will require optimization of reducing or sulfhydryl addition steps and amount of biotinylation reagent.

Materials Required

- Sample Preparation Buffer: 0.1M sodium phosphate, 5mM EDTA, pH 6.0
- 1mL of 4mg/mL (27μM) IgG in Sample Preparation Buffer
- 2-Mercaptoethylamine•HCl (2-MEA), Product No. 20408
- Reaction Buffer: 50mM Tris•HCl, 5mM EDTA, pH 8.0-8.3
- Desalting column: e.g., Dextran Desalting Columns (Product No. 43230)

A. Prepare Reduced IgG

1. Add 1mL of the IgG solution to the vial containing the 6mg 2-MEA (results in 50mM 2-MEA).
2. Mix and incubate the solution for 90 minutes at 37°C.
3. Allow the solution to cool to room temperature. Remove the excess 2-MEA from the reduced IgG using a desalting column equilibrated with Reaction Buffer.

B. Biotinylate Reduced IgG With Iodoacetyl Biotin Reagent

1. Immediately before use, prepare 4mM solution of Iodoacetyl Biotin Reagent:
 - Dissolve 2mg Iodoacetyl-LC-Biotin in 1mL DMF.
 - Dissolve 2.2mg Iodoacetyl-PEG₂-Biotin in 1mL of Reaction Buffer.
2. Add 50µL of the Iodoacetyl Biotin solution per milliliter of the reduced IgG. (This results in 200µM Iodoacetyl Biotin per 50µM reduced hinge-region sulfhydryl groups, corresponding to a 4-fold excess of Iodoacetyl Biotin Reagent.)
3. Mix and incubate reaction in the dark for 90 minutes at room temperature.

Note: Performing the reaction in the dark limits conversion of liberated iodide ions to molecular iodine, which can react with tyrosine residues.⁵
4. Remove non-reacted Biotin Reagent by applying mixture to a desalting column that has been equilibrated with Reaction Buffer. Collect 0.5mL fractions and monitor for the presence of protein by measuring the absorbance at 280nm. The first absorption peak emerging from the column corresponds to fractions containing the biotinylated IgG. Alternatively, the non-reacted Biotin Reagent may be removed by dialysis.

Related Thermo Scientific Products

20357	High Capacity Streptavidin Agarose Resin, 2mL
20290	DTT, 5g
20408	2-Mercaptoethylamine•HCl (2-MEA), 6 × 6mg
20409	TCEP•HCl, 1g
77712	Immobilized TCEP Disulfide Reducing Gel, 5mL
26101	Traut's Reagent, 500mg
26102	SATA, 50mg

Cited References

1. Hermanson, G.T. (1996). *Bioconjugate Techniques*, Academic Press. (Available from Pierce as Product No. 20002).
2. Savage, D.M. *et al.* (1992). *Avidin-Biotin Chemistry: A Handbook*. Pierce Chemical Co., Rockford, IL.
3. Gurd, F.R.N. (1967). Carboxymethylation. *Meth Enzymol* XI, 532-541.
4. Yoshitake, S., *et al.* (1979). Conjugation of glucose oxidase from *Aspergillus niger* and rabbit antibodies using *N*-hydroxysuccinimide ester of *N*-(4-carboxycyclohexyl-methyl)-maleimide. *Eur J Biochem* **101**:395-9.
5. Crestfield, A.M., *et al.* (1963). The preparation and enzymatic hydrolysis of reduced and *S*-carboxymethylated proteins. *J Biol Chem* **238**(2):622-7.

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. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2011 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.