INSTRUCTIONS

EZ-Link® Iodoacetyl-LC-Biotin
EZ-Link® Iodoacetyl-PEG$_2$-Biotin

21333 21334

0254.4

Number	Description
21333	**EZ-Link Iodoacetyl-LC-Biotin (N-Iodoacetyl-N-biotinylhexylenediamine),** 50 mg

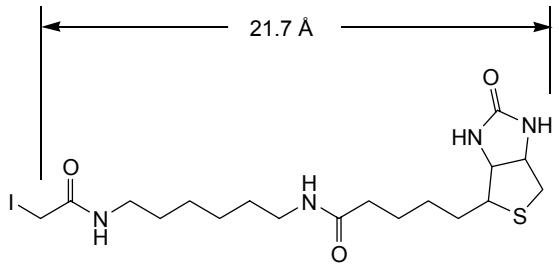
Formula: $C_{18}H_{31}IN_4O_3S$

Molecular Weight: 510.43

Spacer Arm: 27.1 Å

Net Mass Addition: 382.53

Solubility: > 2 mg/ml in DMF for subsequent dilution in water or buffer



21334 **EZ-Link Iodoacetyl-PEG$_2$-Biotin {(+)-Biotinyl-iodoacetamidyl-3,6-dioxaoctanediamine},** 50 mg

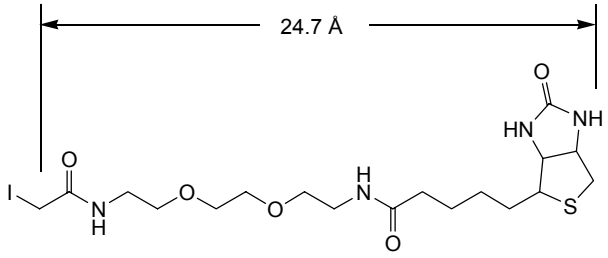
Formula: $C_{18}H_{31}IN_4O_5S$

Molecular Weight: 542.43

Spacer Arm: 24.7 Å

Net Mass Addition: 414.19

Solubility: ≥ 25 mg/ml in water or buffer



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

Introduction

EZ-Link Iodoacetyl-LC-Biotin and Iodoacetyl-PEG$_2$-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-PEG$_2$-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.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermo.com/pierce

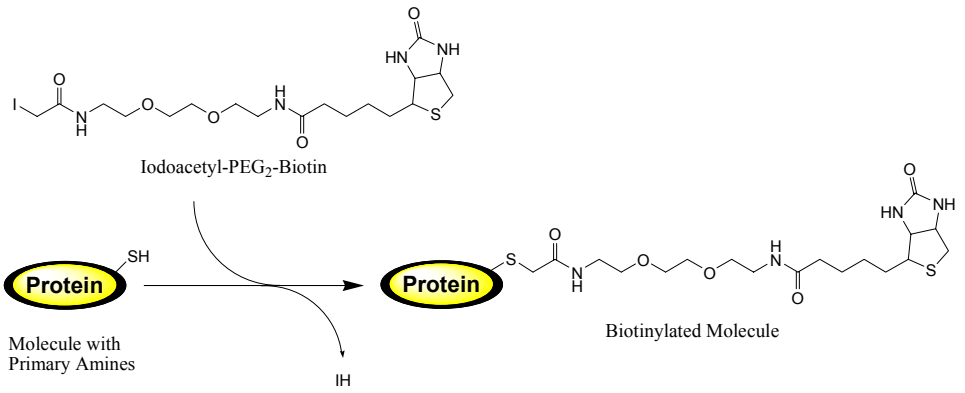


Figure 1. Biotinylation through thioether bond formation using EZ-Link Iodoacetyl-PEG$_2$-Biotin.

Important Product Information

- Iodoacetyl Biotin Reagents are moisture-sensitive. To avoid moisture condensation in the container, equilibrated 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 Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). Reduce disulfide bonds in high molecular weight proteins using 5 mM TCEP (1:100 dilution of Bond-Breaker® TCEP Solution, Product No. 77720) for 30 minutes at room temperature, followed by TCEP removal using a desalting column (e.g., Zeba™ Desalt Spin 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 of 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 50 mM; 1-10 mg 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: 0.1 M sodium phosphate, 5 mM EDTA, pH 6.0
- 1 ml of 4 mg/ml (27 µM) IgG in Sample Preparation Buffer
- 2-Mercaptoethylamine•HCl (2-MEA), Product No. 20408
- Reaction Buffer: 50 mM Tris•HCl, 5 mM EDTA, pH 8.0-8.3
- Desalting column: e.g., D-Salt Dextran Desalting Columns (Product No. 43230)

A. Prepare Reduced IgG

1. Add 1 ml of the IgG solution to the vial containing the 6 mg 2-MEA (results in 50 mM 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 4 mM solution of Iodoacetyl Biotin Reagent:
   - Dissolve 2 mg Iodoacetyl-LC-Biotin in 1 ml DMF.
   - Dissolve 2.2 mg Iodoacetyl-PEG_2-Biotin in 1 ml Reaction Buffer.
2. Add 50 µl of the Iodoacetyl Biotin solution per milliliter of the reduced IgG. (This results in 200 µM Iodoacetyl Biotin per 50 µM 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 ion 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 0.5 ml fractions and monitor for the presence of protein by measuring the absorbance at 280 nm. 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 Products

20290	**DTT,** 5 g
20408	**2-Mercaptoethylamine•HCl (2-MEA),** 6 × 6 mg
20409	**TCEP•HCl,** 1 g
77712	**Immobilized TCEP Disulfide Reducing Gel,** 5 ml
26101	**Traut's Reagent,** 500 mg
26102	**SATA,** 50 mg

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. (available at the Pierce web site as pdf files).
3. Gurd, F.R.N. (1967). Carboxymethylation. *Meth. Enzmol*. 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:**395-99.
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.