INSTRUCTIONS

Maleimide-PEG$_{11}$-Biotin

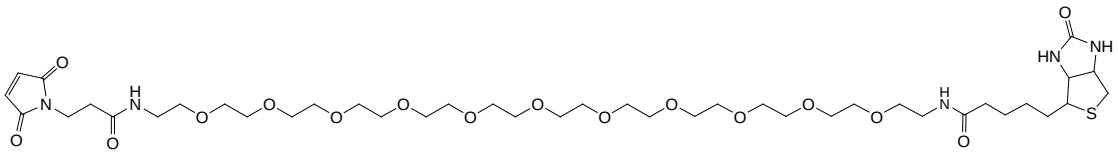
Sulfhydryl-reactive biotin labeling reagent with a long polyethylene glycol (PEG) spacer arm

21911

1894.3

Number	Description
21911	**EZ-Link™ Maleimide-PEG$_{11}$-Biotin,** 25mg

Form: White to off-white, low melting point solid
Molecular Weight: 922.09
Spacer Arm: 51.9Å
Net Mass Addition: 921.46



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

Introduction

Thermo Scientific™ EZ-Link™ Maleimide-PEG$_{11}$-Biotin is a long-chain, water-soluble sulfhydryl-reactive biotinylation reagent. The polyethylene glycol (PEG) spacer arm has a hydrophilic property that is transferred to the final biotin conjugate, which helps to prevent aggregation of labeled antibodies stored in solution. Typical PEG reagents contain heterogeneous mixtures of different PEG chain lengths; however, Thermo Scientific™ Pierce™ PEG reagents are homogeneous compounds of defined molecular weight and spacer arm length, providing precision in optimizing modification applications.

Biotin is a small naturally occurring vitamin that binds with high affinity to avidin and streptavidin proteins. Biotinylated proteins typically retain biological activity because the biotin group is relatively small. An antibody conjugated with several biotin molecules can amplify signal, thereby increasing the sensitivity of many assays. The bond formation between biotin and avidin is rapid and, once formed, is unaffected by most extremes of pH, organic solvents and other denaturing agents. Labeled proteins can be purified using immobilized streptavidin, avidin or Thermo Scientific™ NeutrAvidin™ Protein affinity resins (see Related Thermo Scientific Products) and detected in ELISA, dot blot or Western blot applications.

Maleimide-activated reagents are effective for protein modification of sulfhydryl groups. Maleimide groups react efficiently and specifically with free (reduced) sulfhydryls at pH 6.5-7.5 to form stable thioether bonds (Figure 1). Most proteins have cysteine residues whose side-chain sulfur atoms typically occur in pairs as disulfide bonds. Reduction of these disulfide bonds exposes the sulfhydryl group required as a target for biotinylation with maleimide-activated reagents. Alternatively, sulfhydryl groups can be added to molecules using various modification reagents (see subsequent Important Product Information Section). Maleimide-PEG$_{11}$-Biotin is readily soluble in water or organic solvents such as dimethylsulfoxide (DMSO), methylene chloride or dimethylformamide (DMF).

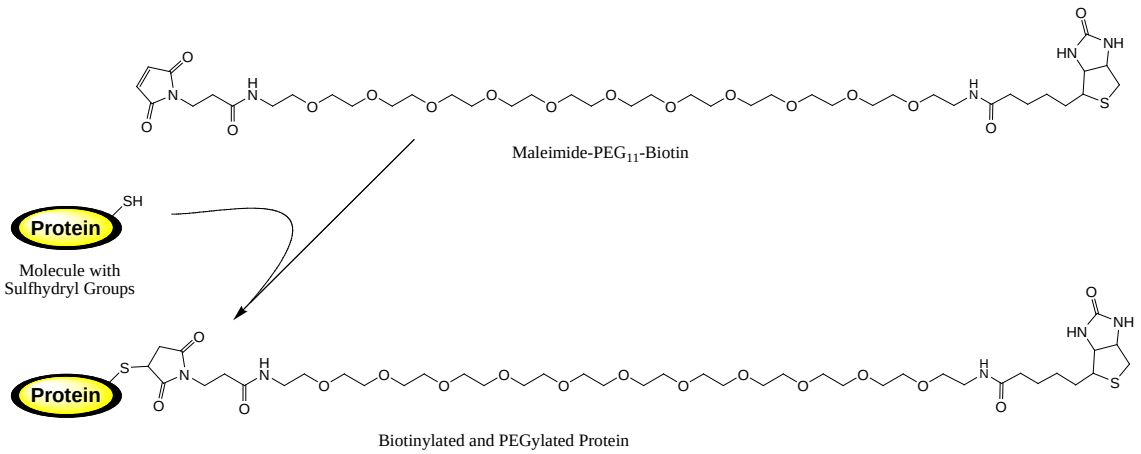


Figure 1. Reaction scheme for biotinylation of sulfhydryl molecules with Maleimide-PEG$_{11}$-Biotin.

Important Product Information

- Maleimide-PEG$_{11}$-Biotin is a low melting-point solid that is difficult to weigh and dispense. To facilitate handling, make a stock solution immediately before first use by dissolving the reagent in dry (anhydrous, molecular sieve-treated) organic solvent, such as dimethylformamide (DMF, Product No. 20673) and dimethylsulfoxide (DMSO, Product No. 20684). Although the maleimide group is more stable than other types of reactive groups, it can hydrolyze to form a non-reactive maleimic acid. Therefore, store unused stock solution in a moisture-free condition (e.g., capped under an inert gas such as argon or nitrogen) at 4°C. Equilibrate reagent vial to room temperature before opening to avoid moisture condensation inside the container. Minimize exposure to air by keeping the stock solution capped by a septum through which reagent can be removed with a syringe. With proper handling, the stock solution is stable for three months.
- Molecules to be reacted with the maleimide 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 5mM TCEP (1:100 dilution of 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.
- Avoid extraneous sulfhydryl-containing components in the reaction buffers during conjugation (e.g., DTT), as they react with the maleimide portion of the reagent, inhibiting and reducing conjugation efficiency of the intended target.
- The maleimide group reacts predominantly with free sulfhydryls at pH 6.5-7.5, forming stable thioether bonds. At pH values > 7.5, reactivity toward primary amines and hydrolysis of the maleimide groups can occur. At pH 7, the maleimide group is ~1000 times more reactive toward a free sulfhydryl than to an amine.
- If desired, excess nonreacted Maleimide-PEG$_{11}$-Biotin can be removed by size exclusion using either desalting columns or dialysis units (see Related Thermo Scientific Products).

Additional Materials Required

- Water-miscible organic solvent (molecular sieve-treated) such as dimethylsulfoxide (DMSO, Product No. 20684) or dimethylformamide (DMF, Product No. 20673) for preparing reagent stock solution
- Small-volume, non-coring syringes for dispensing reagent stock solution while minimizing exposure to the air
- Phosphate-buffered saline (PBS) or other sulfhydryl-free buffer having pH 6.5-7.5 for use as reaction buffer (see Important Product Information and Related Thermo Scientific Products)
- Desalting columns or dialysis units for buffer exchange and removal of excess reagent following modification (e.g., Zeba Spin Desalting Columns or Thermo Scientific™ Slide-A-Lyzer™ Dialysis Units)

Procedure for Biotinylating Proteins with Maleimide-PEG$_{11}$-Biotin

The amount of Maleimide-PEG$_{11}$-Biotin to use for each reaction depends on the number of free sulfhydryls, the amount of modification desired, and the amount and concentration of the molecule to be labeled. By regulating the reagent-to-target molar ratio in the reaction, the extent of labeling can be controlled. As a starting point use a 5- to 20-fold molar excess of reagent for protein solutions > 2mg/mL. When labeling more dilute solutions, a greater relative molar fold excess of reagent may be necessary to achieve the same results. Optimal molar ratios for small molecule modification may differ significantly. Example calculations for IgG modification (molecular weight 150,000) are provided for convenience.

A. Calculate the Amount of Reagent Needed

1. Calculate the quantity in millimoles of the reagent to add to the reaction for a 20-fold molar excess:

$$\text{mL protein} \times \frac{\text{mg protein}}{\text{mL protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{\text{20 mmol Biotin Reagent}}{\text{mmol protein}} = \text{mmol Biotin Reagent}$$

   Note: the value 20 in this equation corresponds to the suggested reagent molar fold excess for a 2mg/mL protein sample.

2. Calculate microliters of 250mM Biotin Reagent Stock Solution (prepared in Step B.1) to add to the reaction:

$$\text{mmol Biotin Reagent} \times \frac{1{,}000{,}000\ \mu\text{L}}{\text{L}} \times \frac{\text{L}}{250\ \text{mmol}} = \mu\text{L Biotin Reagent Stock Solution}$$

Example Calculation:

For 1mL of a 2mg/mL IgG (150,000 MW) solution, ~1µL of 250mM Biotin Reagent will be added.

$$1\,\text{mL IgG} \times \frac{2\,\text{mg IgG}}{1\,\text{mL IgG}} \times \frac{1\,\text{mmol IgG}}{150{,}000\,\text{mg IgG}} \times \frac{20\,\text{mmol Biotin Reagent}}{1\,\text{mmol IgG}} = 0.000266\,\text{mmol Biotin Reagent}$$

$$0.000266\,\text{mmol Biotin Reagent} \times \frac{1{,}000{,}000\,\mu\text{L}}{\text{L}} \times \frac{\text{L}}{25\,\text{mmol}} = 1.07\,\mu\text{L of 250 mM Biotin Reagent Stock Solution}$$

B. Prepare 250mM Reagent Stock Solution

1. Read the Important Product Information (previous section) before preparing and storing this solution.
2. Remove vial of reagent from 4°C storage and fully equilibrate it to room temperature before opening.
3. Prepare a 250mM Biotin Reagent Stock Solution by dissolving all 25mg of reagent (i.e., entire contents of vial, approximately 25µL) in 84µL of dry water-miscible solvent (e.g., DMF or DMSO).
4. Cap, store and handle stock solutions as directed in the Important Product Information Section.

C. Labeling Reaction

1. Dissolve protein to be modified in sulfhydryl-free buffer at pH 6.5-7.5, according to the calculations made in Section A.

   Note: Protein already in sulfhydryl-free buffer at pH 6.5-7.5 may be used without buffer exchange or dilution.

2. If the vial of Biotin Reagent Stock Solution had been stored since preparation, remove it from 4°C storage and fully equilibrate it to room temperature before opening.
3. Using a syringe, remove an appropriate volume (see Calculations in Section A) of 250mM Biotin Reagent Stock Solution, dispense it into the protein solution and mix well.
4. Incubate reaction on ice or room temperature for two hours to overnight.

   Note: Except for possible degradation or microbial growth, there is no harm in reacting longer than the specified time.

5. Labeling is complete at this point and, although excess nonreacted and hydrolyzed Biotin Reagent remains in the solution, it is often possible to perform preliminary tests of the labeled protein. Once proper function and labeling has been confirmed, the labeled protein may be purified from nonreacted Maleimide-PEG$_{11}$-Biotin by desalting or dialysis.

Troubleshooting

Problem	Possible Cause	Solution
Protein is not biotinylated	No free sulfhydryls available	Reduce existing disulfide bonds to generate free sulfhydryls, or introduce sulfhydryls with Traut's Reagent, SATA or SAT(PEG)$_4$
	Maleimide group is hydrolyzed and non-reactive	Do not store reagent in aqueous solutions or solvent that has absorbed water

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 Pierce Biotin Quantitation Kit (Product No. 28005) contains a premix of HABA and avidin and a biotinylated protein control supplied in convenient Thermo Scientific™ No-Weigh™ Microtube packaging, which eliminates the difficulties associated with weighing small quantities of reagent.

Related Thermo Scientific Products

28005 **Pierce Biotin Quantitation Kit**

21902 **No-Weigh Maleimide-PEG$_2$-Biotin,** 8 × 2mg microtubes

21901 **EZ-Link Malemide-PEG$_2$-Biotin,** 50mg

21362 **EZ-Link NHS-PEG$_4$-Biotin,** 50mg

21312 **EZ-Link NHS-PEG$_{12}$-Biotin,** 25mg

21126 **Streptavidin, Horseradish Peroxidase Conjugated,** 1mg

15120 **Streptavidin Coated Plates**, 5 plates (see website for a complete listing of plate products)

20347 **Streptavidin Agarose Resin**, 2mL (see website for a complete listing of related products)

20228 **Pierce Monomeric Avidin Kit**, bind and gently elute biotin-labeled molecules

28372 **BupH Phosphate Buffered Saline Packs,** 40 pack

66382, 66807 **Slide-A-Lyzer Dialysis Cassette Kits,** 3mL and 12mL sample volumes, respectively

89891 **Zeba Spin Desalting Columns, 5mL,** 5 × 5mL columns

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