INSTRUCTIONS

EZ-Link BMCC-Biotin

MAN0016375
Rev. A.0
Pub. Part No. 2160466.4

21900

Number	Description
21900	**EZ-Link BMCC-Biotin,** 1-Biotinamido-4-[4´-(maleimidomethyl)cyclohexanecarboxamido]butane

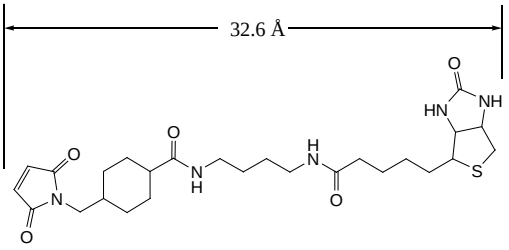
Quantity: 50mg

Formula: $C_{26}H_{39}N_5O_5S$

Molecular Weight: 533.68

Spacer Arm Length: 32.6Å

Solubility: ~4.5mg/mL (8.5mM) in DMSO. May require heating at 37°C for up to 10 minutes with intermittent mixing



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

Introduction

Thermo Scientific™ EZ-Link™ BMCC-Biotin is a sulfhydryl-reactive biotinylation reagent with a long spacer arm. 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). BMCC-Biotin is not directly soluble in water or aqueous buffers; however, if it is first dissolved in dimethylsulfoxide (DMSO) or other organic solvent, then it can be diluted and remain soluble in an aqueous buffer at typical reaction concentrations.

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 captured or detected in ELISA, dot blot or Western blot applications using immobilized or conjugate products containing streptavidin, avidin or NeutrAvidin™ Protein (see Related Thermo Scientific Products).

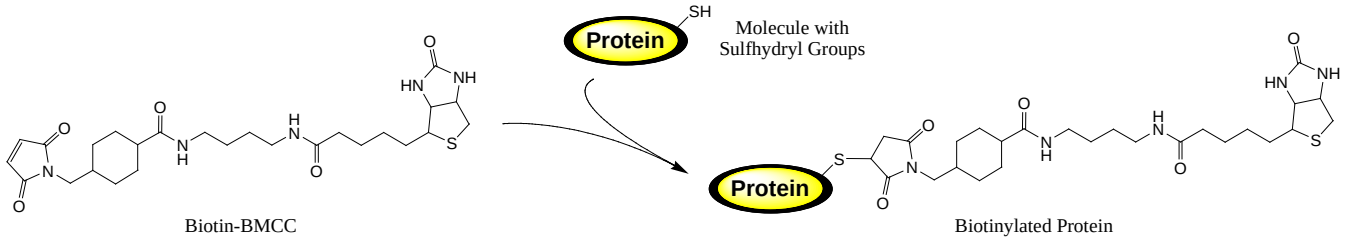


Figure 1. Reaction scheme for biotinylation of sulfhydryl molecules with BMCC-Biotin.

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Important Product Information

- BMCC-Biotin is moisture-sensitive. Store product in the original container at 4°C with desiccant. Equilibrate vial to room temperature before opening to avoid moisture condensation onto the product. Prepare reagent solution immediately before use. The maleimide moiety will hydrolyze and become non-reactive in water; therefore, aqueous stock solutions cannot be prepared for storage. Discard any unused reconstituted reagent.
- Molecules to be reacted with the maleimide 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.
- 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.

Additional Materials Required

- 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)
- Dimethylsulfoxide (DMSO), Product No. 20688 (see 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 BMCC-Biotin

The optimal amount of BMCC-Biotin to use for each reaction depends on a number of factors. By regulating the reagent-to-target molar ratio in the reaction, the extent of labeling can be controlled. As a starting point use a 10- to 30-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. Calculations

1. Calculate the quantity in millimoles of the reagent to add to the reaction for a 10-fold molar excess:

$$\text{mL protein} \times \frac{\text{mg protein}}{\text{mL protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{10\ \text{mmol Biotin Reagent}}{\text{mmol protein}} = \text{mmol Biotin Reagent}$$

Note: the value 10 in this equation corresponds to the suggested reagent molar fold excess for a 2mg/mL protein sample.

2. Calculate microliters of 8mM Biotin Reagent Stock Solution (prepared in Step B.1) to add to the reaction:

$$\text{mmol Biotin Reagent} \times \frac{1{,}000{,}000\ \mu\text{L}}{\text{L}} \times \frac{\text{L}}{8\ \text{mmol}} = \mu\text{L Biotin Reagent Stock Solution}$$

Example: For 1 mL of a 2mg/mL IgG (150,000 MW) solution, ~17µL of 8mM Biotin Reagent will be added.

$$1\ \text{mL IgG} \times \frac{2\ \text{mg IgG}}{1\ \text{mL IgG}} \times \frac{1\ \text{mmol IgG}}{150{,}000\ \text{mg IgG}} \times \frac{10\ \text{mmol Biotin Reagent}}{1\ \text{mmol IgG}} = 0.000133\ \text{mmol Biotin Reagent}$$

$$0.000133\ \text{mmol Biotin Reagent} \times \frac{1{,}000{,}000\ \mu\text{L}}{\text{L}} \times \frac{\text{L}}{8\ \text{mmol}} = 16.6\ \mu\text{L of 8 mM Biotin Reagent Stock Solution}$$

B. Biotin 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. Immediately before use, add 500µL of DMSO to 2.1mg of BMCC-Biotin to prepare an 8mM stock solution.
3. Add the appropriate volume of the BMCC-Biotin (see Calculations section) to the protein solution and mix.
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 with the labeled protein. Once proper function and labeling has been confirmed, the labeled protein may be purified from nonreacted BMCC-Biotin by desalting or dialysis.

Troubleshooting

Problem	Possible Cause	Solution
Protein is not biotinylated	There were 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 was hydrolyzed and non-reactive	Do not store reagent in aqueous solutions or solvent that has absorbed water

Additional Information

A. 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 and avidin and a biotinylated protein control supplied in convenient No-Weigh™ Microtube packaging.

B. Example Labeling and Application Data

Goat anti-mouse IgG (Product No. 31160) was biotinylated overnight with BMCC-Biotin according to the protocol using 25:1 and 50:1 molar ratios of BMCC-Biotin to IgG. The IgG was also biotinylated using 50:1 and 100:1 molar ratios of Iodoacetyl-LC-Biotin (Product No. 21333). To test function of the labeled antibodies, an ELIFA (enzyme-linked immunoflow assay) of purified mouse IgG was performed using a Thermo Scientific Easy-Titer ELIFA System. Nitrocellulose membrane was coated with 200µL of mouse IgG (Product No. MG100) at 10µg/mL in Dulbecco's PBS (Product No. 28374) and then blocked with 200µL of Thermo Scientific™ SuperBlock™ Blocking Buffer (Product No. 37515). Each test biotinylated antibody was serially diluted and cycled through different membrane wells. After washing with PBS, the membrane was probed with 200µL of a 1/2500 dilution of Thermo Scientific Streptavidin-HRP (Product No. 21126) in SuperBlock Blocking Buffer containing 0.05% Tween™-20 Detergent. After extensive washing, the membrane was treated with 200µL of Thermo Scientific 1-Step Slow TMB (Product No. 34024) and then 50µL of 1M sulfuric acid. Absorbance of each well was measured at 450nm and plotted in Figure 2.

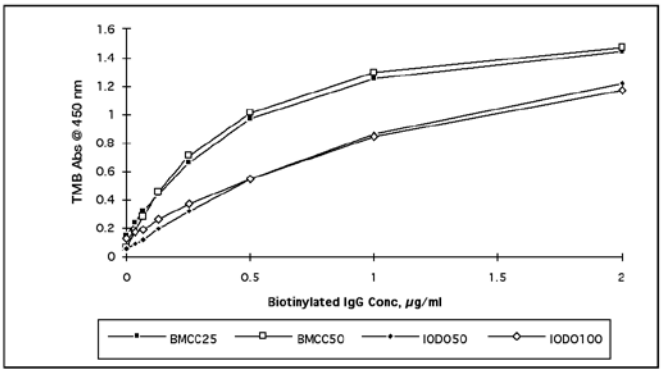

Figure 2. Biotinylation with BMCC-Biotin produces more effective detection antibody than biotinylation with Iodoacetyl-LC-Biotin.

Related Thermo Scientific Products

28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
20688	**Dimethylsulfoxide (DMSO),** 50mL
66382	**Slide-A-Lyzer™ Dialysis Cassette Kit, 10K MWCO, 3mL**
89891	**Zeba Spin Desalting Columns, 7K MWCO, 5mL**
28005	**Pierce™ Biotin Quantitation Kit**
21901	**EZ-Link Malemide-PEG_2-Biotin,** 50mg
21911	**EZ-Link Malemide-PEG_{11}-Biotin,** 25mg
21362	**EZ-Link NHS-PEG_4-Biotin,** 50mg
21126	**Streptavidin, Horseradish Peroxidase Conjugated,** 1mg
15120	**Streptavidin Coated Plates, 96-well strip, clear,** 5/pkg
20347	**Streptavidin Agarose Resin**, 2mL
20228	**Pierce Monomeric Avidin Agarose,** 5mL

Product References

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