ChromaLink Biotin Maleimide

$C_{40}H_{53}N_9O_9S$, Mol. Wt: 836.0

Catalog# B-1012

Introduction to ChromaLink Labeling Technology

ChromaLink Biotin Maleimide incorporates UV-traceable biotin onto thiol containing proteins, peptides and/or antibodies. ChromaLink Biotin Maleimide has been engineered to include many novel features. As illustrated in Figure 1, the molecule's structure contains a bis-arylhydrazone chromophore ***(a)***, linked by a PEG3 linker arm ***(b)***, to biotin ***(c)***. This reagent permits direct spectroscopic quantification of incorporated biotin. The extended PEG3 linker preserves biotin/streptavidin affinity and maintains protein solubility after modification while the maleimido functional group ***(d),*** efficiently modifies thiols in aqueous buffers.

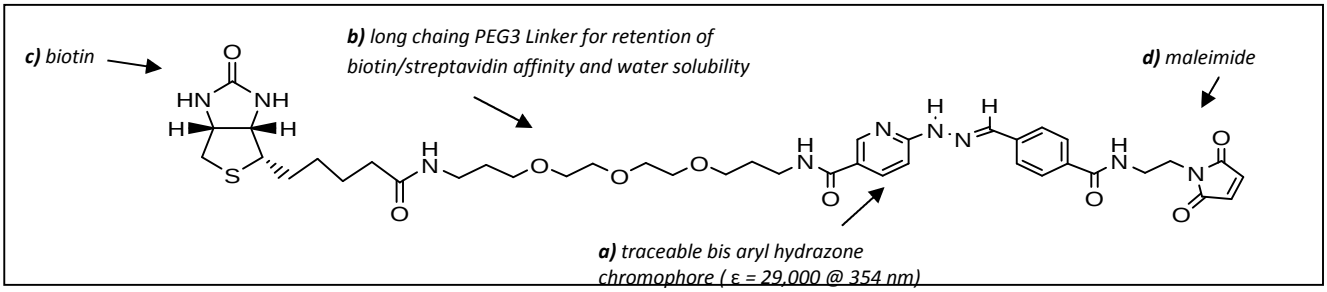

Figure 1. Molecular structure of ChromaLink Biotin Maleimide

Labeling of proteins with ChromaLink Biotin eliminates the need to carry out cumbersome and time-consuming HABA assays often employed to quantify biotin incorporation. Instead, biotin incorporation is quantified by means of a simple spectrophotometric measurement at two wavelengths (A280 / A354). Typical labeling results are illustrated in Figure 2 by spectral overlay scans of four samples. As illustrated, Bovine Serum Albumin (100 ul @ 1 mg/ml) was labeled at 0, 5, 10, and 20 mole equivalents using ChromaLink Biotin Maleimide. Spectral analysis illustrates how easy it is to visualize, confirm, and quantify biotin incorporation.

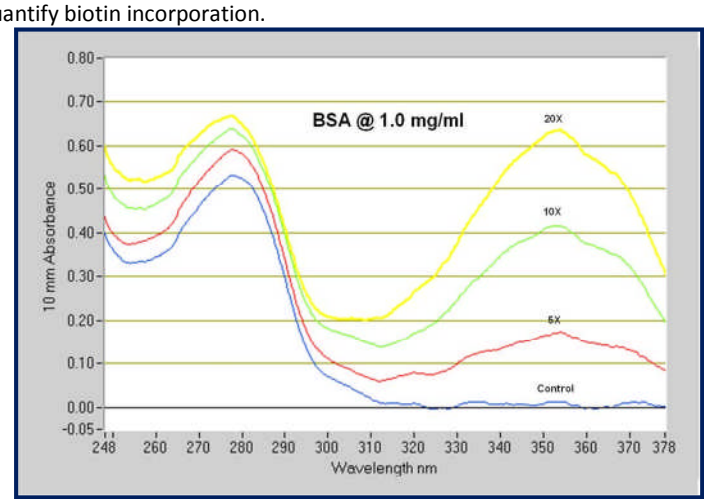

Figure 2. Superimposed spectra of BSA biotinylated using ChromaLink Biotin Maleimide. Various biotin-to-protein mole equivalents (5X, 10X and 20X) were used. Note the UV-signature at 354nm indicating incorporation of biotin. All spectra were scanned on a Molecular Dynamics SpectraMax PlusTM UV-VIS plate reader (220-420 nm).

Methods

Note: This protocol and any documents linked below can be downloaded from the appropriate category in the Solulink Library at http://www.solulink.com/library.

Additional Materials Required

Reagents

Zeba™ Desalt spin columns (cat # S-4004-025)
Modification Buffer pH6.5 (cat # S-4003-005)
10mM TCEP·HCl in water (MW 286.65)
Elution Buffer (based on final assay)
DMF anhydrous (cat # S-4001-005)
Albumin Standard, 2 mg/ml (Pierce Chemicals, #23209)
BCA Protein Assay Kit (Pierce Chemicals, #23225) or Bradford (BioRad, #500-0006)

Equipment

Variable-speed bench-top centrifuge
Spectrophotometer, Plate Reader, or NanoDrop

Modification Procedure

A. Desalting procedure

1. Desalt/buffer exchange the protein into Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 8.0); if needed, refer to the **desalting protocol**.

Notes:

a) Buffer exchange removes all small molecule contaminants, from the protein solution before modification.
b) Do not use PBS. High-level buffering capacity, *i.e.* 100 mM phosphate, is necessary for successful modification.
c) For desalting proteins SolulinK recommends Zeba Desalt Spin columns (Pierce, # 89882) available through SoluLink.

B. Determine the concentration of the desalted protein

1. Determine the concentration of the protein to be modified using a **Bradford assay** (BioRad, #500-0006) or the **BCA assay** (ThermoScientific, #23223). Alternatively the A280 can be used if the protein extinction coefficient is known (E1%).
2. Adjust the concentration to 1-5 mg/mL in Modification Buffer pH 8.0, if necessary.

C. Prepare a ChromaLink Biotin Maleimide /DMF stock solution

1. Prepare a stock solution of ChromaLink Biotin Maleimide in anhydrous DMF (or DMSO) by dissolving 1-4 mg of ChromaLink in 100 µL anhydrous DMF.

Note:

The ChromaLink Biotin/DMF stock solution can be stored for up to 1 week at -20°C if prepared with anhydrous DMF (SolulinK catalog # S-4000).

D. Biotinylation of the protein

1. Add 1/10th volume of freshly prepared 10mM TCEP-HCl in molecular grade water to the buffer exchanged thiol protein; the protein being modified already contains free thiols, this step is not required.
2. With the aid of the **ChromaLink Biotin Maledimide Reagent Calculators (Tab 1)**, add 10-20 molar equivalents of ChromaLink Biotin Maleimide stock solution to the protein solution.
3. Allow reaction to incubate at room temperature for 1 hour.

E. Desalting procedure

1. Desalt/buffer exchange the biotinylated protein into your buffer of choice as directed in part A; if needed, refer to the **Desalting Protocol**.

F. Quantify biotin molar substitution ratio

a. A280/354 Method

1. Take a UV spectra of the biotin labeled protein. Record the A280 and A354.
2. The biotin incorporation (molar substitution ratio (MSR)) can be determined using the **ChromaLink Biotin Maledimide Reagent Calculators (Tab 2)** by plugging in the absorbance peaks at A280 and A354. For optimal labeling, the biotin MSR should be between 3-8, depending on the size of the protein.

b. A354 - Bradford or BCA Method

1. Determine the concentration of the biotin labeled protein as in part B.
2. Determine the A354 using a spectrophotometer
3. The biotin incorporation (molar substitution ratio (MSR)) can be determined using the **ChromaLink Biotin Maledimide Reagent Calculators (Tab 3)** by inserting the protein concentration and the Absorbance peaks at A280 and A354. For optimal labeling, the biotin MSR should be between 3-8, depending on the size of the protein.

The protein is now biotin labeled and ready for conjugation to streptavidin coated molecules or surfaces.

Troubleshooting

Problem	Possible Cause	Solution
Protein was not biotin labeled or poorly labeled.	Protein was not sufficiently reduced using TCEP	React the Protein with TCEP at the same time as the reaction with the Chromalink Maleimide Biotin
ChromaLink Biotin Maleimide was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the maleimido group	Do not store the ChromaLink Biotin Maleimide for more than 1 week in wet DMF solvents
Molar substitution readings are out of detectable range	Protein concentrations are out of recommended range	Concentrate or dilute protein samples into recommend range
Precipitation of protein on modification	Precipitation of biotin modified proteins may occur due to a drastic change in the isoelectric properties of the modified protein.	After the biotinylation reaction is complete, addition of 1M Tris (pH 9.0) can sometimes be used to resuspend the biotinylated protein by adjusting the pH above the pI of the protein

Stability

ChromaLink Biotin labeled proteins are stable. The Chromalink bond is sensitive to strong nucleophiles and oxidizing agents.

Related Solulink Products

S-4025-010 Zeba™ Desalt spin columns	S-4001-005 DMF anhydrous	M-1002-010 NanoLink Streptavidin Magnetic Beads
S-4003-005 Modification Buffer	S-9007-105K ChromaLink Biotin Labeling Kit	M-1003-010 MagnaLink Streptavidin Magnetic Beads