

## Introduction to ChromaLink Labeling Technology

ChromaLink Biotin incorporates UV-traceable biotin onto proteins and antibodies. ChromaLink Biotin 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 succinimidyl ester functional group (**d**), efficiently modifies lysines in aqueous buffers.

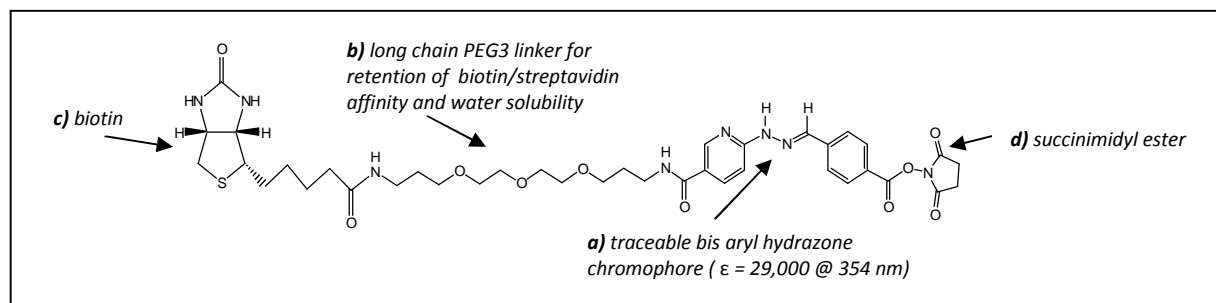


Figure 1. Molecular structure of ChromaLink Biotin

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 IgG (100  $\mu$ l @ 5 mg/ml) was labeled at 0, 5, 10, and 15 mole equivalents using ChromaLink Biotin. Spectral analysis illustrates how easy it is to visualize, confirm, and quantify biotin incorporation.

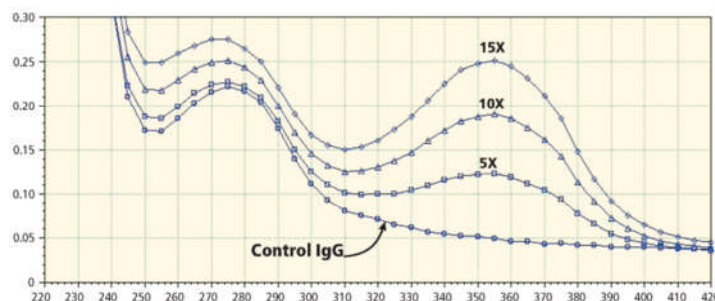


Figure 2. Superimposed spectra of bovine IgG biotinylated using ChromaLink Biotin. Various biotin-to-protein mole equivalents (5X, 10X and 15X) were used. Note the UV-signature at 354nm indicating incorporation of biotin. All spectra were scanned on a Molecular Dynamics SpectraMax Plus™ UV-VIS plate 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 (cat # S-4003-005)  
 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

- 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 free amine-containing contaminants, *e.g.* tris, glycine, 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 1-2.5 mg/mL in Modification Buffer pH 8.0, if necessary.

**C. Prepare a ChromaLink Biotin/DMF stock solution**

1. Prepare a stock solution of ChromaLink Biotin in anhydrous DMF (or DMSO) by dissolving 1-4 mg of ChromaLink in 100  $\mu$ L anhydrous DMF.

**Note:**

The ChromaLink Biotin/DMF stock solution can be stored for 2 weeks at -20°C if prepared with anhydrous DMF (Solulink catalog# S-4001).

**D. Biotinylation of the protein**

1. With the aid of the [ChromaLink Biotin Protein Labeling Calculator](#), and add 10-20 molar equivalents of ChromaLink Biotin stock solution to the protein solution.
  - a. **Note:** be sure to use the correct values for Chromalink Biotin in the **Reagent Information** section of the calculator.
2. Allow reaction to incubate at room temperature for 90 minutes.

**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 [Protein 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 Solulink's [E1% Biotin MSR Calculator](#) 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 Solulink's [BCA/Bradford Assay Biotin MSR Calculator](#) 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 has been contaminated with amine containing compounds	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration device
	The concentration of the protein was too low	Increase the concentration of the protein to >2.0 mg/mL
ChromaLink Biotin was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester	Use a good quality anhydrous DMF/DMSO to solubilize ChromaLink.
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 over-modification of available lysines and 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
		Reducing the number of equivalents can sometimes prevent precipitation but it will also reduce the MSR
	Over-modification of the protein	Recheck the concentration of CLB working stock used to label 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 Zeba™ Desalt spin columns  
S-4003 Modification Buffer

S-4001 DMF (Anhydrous)  
S-9007-105K ChromaLink Biotin Labeling Kit

M-1002 NanoLink Streptavidin Magnetic Beads  
M-1003 MagnaLink Streptavidin Magnetic Beads

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