Sulfo ChromaLink Biotin (Water Soluble)

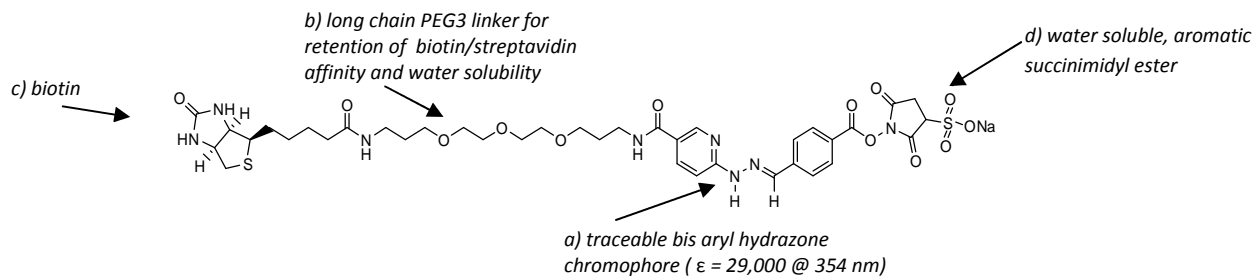$C_{38}H_{49}N_8NaO_{13}S_2$; Mol. Wt.: 912.96

Catalog# B-1007

Introduction to ChromaLink Labeling Technology

ChromaLink Biotin incorporates UV-traceable biotin onto proteins containing lysine residues (amine groups) via a water-soluble-succinimidyl activated ester.ChromaLink Biotin has been engineered to include many novel features. As illustrated in Figure 1, the molecule's structure contains a bis-aryl hydrazonechromophore **(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.

b) long chain PEG3 linker for retention of biotin/streptavidin affinity and water solubility

d) water soluble, aromatic succinimidyl ester

c) biotin

a) traceable bis aryl hydrazone chromophore (ε = 29,000 @ 354 nm)

Figure 1. Molecular structure of ChromaLink Biotin (water soluble)

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 ul @ 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.
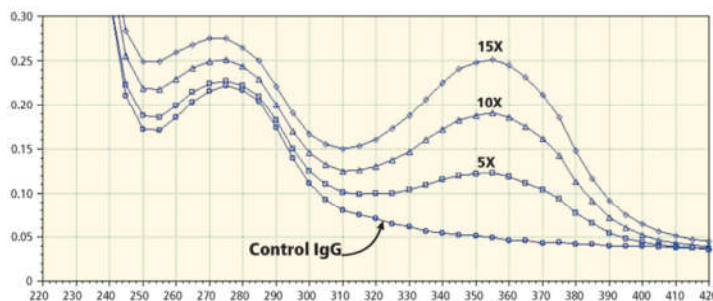
Figure 2. Superimposed spectra of bovine IgG biotinylated using ChromaLink Biotin. Various biotin-to-protein mole equivalents (5X, 10X and 15X) were used. Note the traceable UV-signature @ 354nm indicating incorporation of biotin. All spectra were scanned on a Molecular Dynamics SpectraMax PlusTM UV-VIS 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-4004-010)
10X Modification Buffer (cat # S-4000-005)
Elution Buffer (based on final assay)
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 microcentrifuge
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).

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) also 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) or spectrophotometrically if the extinction coefficient of the protein is known.
2. Bring the concentration to 1-2 mg/mL in Modification Buffer pH 8.0

C. Prepare a ChromaLink Biotin/water stock solution

1. Enter the required information into the **Sulfo ChromaLink Biotin (Water-Soluble) Calculators (Tab 1)**.
2. Prepare a stock solution of Sulfo ChromaLink Biotin by dissolving the required amount of Sulfo ChromaLink Biotin in the designated amount of molecular biology grade water at the concentration determined by the calculator.
3. Pipette and vortex vigorously to ensure complete and thorough dissolution.

Note:
The ChromaLink Biotin is not stable long term in aqueous solution and must be used immediately after preparation.

D. Biotinylation of the protein

1. With the aid of the calculator, depending on protein concentration, add 15 or 20 molar equivalents of Sulfo ChromaLink Biotin stock solution to the protein solution, and mix thoroughly.
2. Allow reaction to incubate at room temperature for 120 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 **Sulfo ChromaLink Biotin (Water-Soluble) 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 MSR can be determined using **Sulfo ChromaLink Biotin (Water-Soluble) Calculators (Tab 3)** by inserting the protein concentration and the absorbance peaks at 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	The reagent was left for more than 1 hour in aqueous solution	Use the ChromaLink stock solution immediately after preparation
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
	Over-modification of the protein	Reducing the number of equivalents can sometimes prevent precipitation but it will also reduce the MSR
		Recheck the concentration of SCLB stock solution 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-4003-005 Modification Buffer   M-1003-010 MagnaLink Streptavidin Magnetic Beads