USER GUIDE

Sulfo ChromaLINK® Biotin (Water Soluble)

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

Together we breakthrough™

Cat. No. B-1007

Storage Desiccated: -15° to -25°C

Introduction to ChromaLINK Labeling Technology

ChromaLINK Biotin incorporates UV-traceable biotin onto proteins containing lysine residues (amine groups) via a water soluble succinimidyl ester. ChromaLINK Biotin has been engineered to include many novel features. As illustrated in Figure 1, the molecule's structure contains a bis-aryl hydrazone 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 sulfo succinimidyl ester functional group (d) efficiently modifies lysines in aqueous buffers.

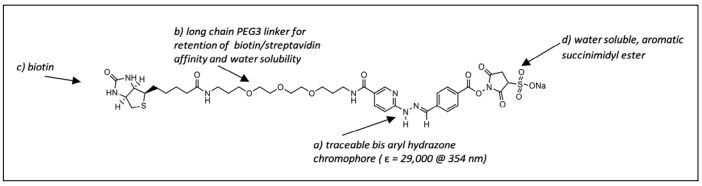

Figure 1. Molecular structure of Sulfo ChromaLINK Biotin (water soluble).

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 (A_{280}/A_{354}). Typical labeling results are illustrated in Figure 2 by spectral overlay scans of four samples. As illustrated, Bovine IgG (100 µl at 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.

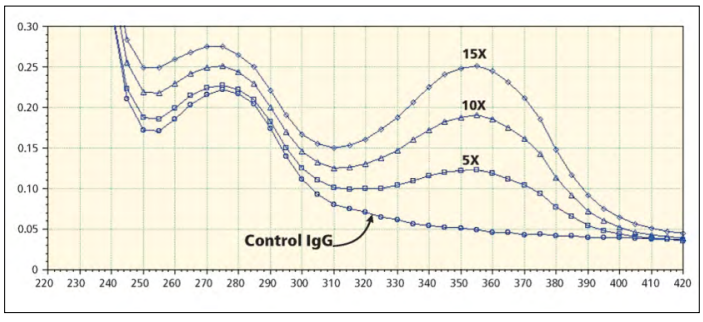

Figure 2. Superimposed spectra of bovine IgG biotinylated using ChromaLINK Biotin. Various biotin-to-protein mole equivalents (5X, 10X, and 15X) were used. Note UV-signature at 354 nm indicating incorporation of biotin. All spectra were scanned on a SpectraMax Plus™ UV-Vis plate reader (220–420 nm).

Additional materials required

Reagents

Thermo Scientific™ Zeba™ Desalting Columns

Modification Buffer (10X)

Elution Buffer (based on final assay)

Albumin Standard, 2 mg/ml

BCA Protein Assay Kit or Bradford Assay Kit

Equipment

Variable-speed bench-top centrifuge

Spectrophotometer, Plate Reader, or NanoDrop™

Modification Procedure

A. Desalting

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

   Notes:

   a) Buffer exchange removes all free amine-containing contaminants, e.g. tris or 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, Zeba Desalting Columns are recommended.

B. Determine the concentration of the desalted protein

1. Determine the concentration of the protein to be modified using a spectrophotometer and the known E1% (280 nm). Alternatively, a Bradford assay or BCA assay can be used if the protein extinction coefficient is not known.
2. Adjust the concentration to 1.0–2.5 mg/ml in 1X Modification Buffer, pH 8.0, if necessary.

C. Prepare a Sulfo ChromaLINK Biotin/buffer stock solution

1. Prepare a stock solution of ChromaLINK Biotin in aqueous buffer by dissolving 1–4 mg of ChromaLINK Biotin in 100 µL of 1X Modification Buffer.

   Note: ChromaLINK Biotin is not stable in aqueous solution and must be used immediately after preparation.

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D. Biotinylation of the protein

1. With the aid of the Sulfo ChromaLINK Biotin Protein Labeling Calculator, add 10–20 mole equivalents of Sulfo ChromaLINK Biotin stock solution to the protein solution.
2. Immediately pipet up and down, then gently vortex to mix.
3. Allow reaction to incubate at room temperature for 90 minutes.

E. Desalting procedure

1. Desalt/buffer exchange the biotinylated protein into the buffer of choice as directed in part A.

F. Quantify biotin molar substitution ratio (MSR)

A280/A354 Method

1. Take a UV spectra of the biotin-labeled protein. Record the A_{280} and A_{354}.
2. Biotin incorporation (molar substitution ratio, or MSR) can be determined using the E1% ChromaLINK Biotin MSR Calculator by entering the required information. For optimal labeling, the biotin MSR should be between 3–8 for an antibody (150 kDa).

A354 – Bradford or BCA Method

1. Determine the concentration of the biotin-labeled protein as in part B if the extinction coefficient is not known.
2. Determine the A_{354} using a spectrophotometer.
3. Biotin incorporation (molar substitution ratio, or MSR) can be determined using the BCA Assay ChromaLINK Biotin MSR Calculator by inserting the protein concentration and the absorbance at 354 nm. For optimal labeling, the biotin MSR should be between 3–8 for an antibody (150 kDa).

The protein is now biotinylated and ready for use.

Application Notes

Protein Desalting Protocol

Oligo Desalting Protocol

Performing a Bradford assay

Performing a BCA protein assay

Troubleshooting Guide

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