USER GUIDE

ChromaLINK® Biotin Maleimide

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

Together we breakthrough™

Cat. No. B-1012-010

Storage Desiccated: -15° to -25°C

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-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 maleimide functional group (d) efficiently modifies thiols in aqueous buffers.

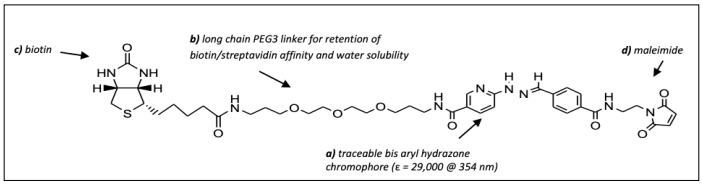

Figure 1. Molecular structure of ChromaLINK Biotin Maleimide.

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 (A_{280}/A_{354}). Typical labeling results are illustrated in Figure 2 by spectral overlay scans of four samples. As illustrated, TCEP-reduced IgG2a antibody (100 µl @ 1 mg/ml) was labeled at 0, 5, 10, and 15 mole equivalents using ChromaLINK Biotin Maleimide. Spectral analysis illustrates how easy it is to visualize, confirm, and quantify biotin incorporation.

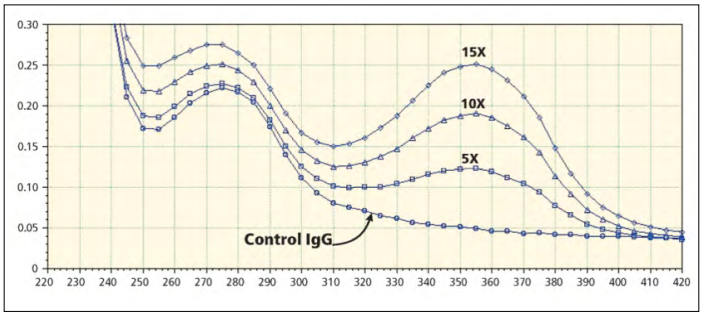

Figure 2. Superimposed spectra of biotinylated reduced IgG2a using ChromaLINK Biotin Maleimide. Various biotin-to-protein mole equivalents (5X, 10X, and 15X) were used. Note the UV-signature at 354 nm indicating incorporation of biotin. All spectra were scanned on a SpectraMax Plus™ UV-Vis plate reader (220–420 nm).

Additional materials required

Reagents

Thermo Scientific™ Zeba™ Desalting columns

Maleimide Modification Buffer, pH 6.5

10 mM TCEP•HCl in water (MW 286.65)

Elution Buffer (based on final assay)

Anhydrous DMF

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 Maleimide Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 6.5).

   Notes:

   a) Buffer exchange removes all small molecule contaminants from the protein solution before modification and exchanges the protein into the correct buffer.

   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 Maleimide Modification Buffer, pH 6.5, 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 Maleimide in 100 µl of anhydrous DMF.

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D. Biotinylation of the protein

1. If the protein requires reduction, add one tenth volume of freshly prepared 10 mM TCEP in ultrapure water to the buffer exchanged protein sample. Other reducing agents such as DTT or MEA may also be used.
2. With the aid of the ChromaLINK Biotin Maleimide Protein Labeling Calculator, add 10–20 mole 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 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 required information into the calculator. 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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