

Introduction to ChromaLink™ Labeling Technology

Sulfo ChromaLink Digoxigenin (DIG) incorporates UV-traceable DIG onto proteins containing lysine residues (amine groups) via a water-soluble-succinimidyl activated ester. Sulfo ChromaLink DIG 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 digoxigenin **(c)**. This reagent permits direct spectroscopic quantification of incorporated digoxigenin. The extended PEG3 linker preserves DIG/anti-DIG antibody affinity and maintains protein solubility after modification while the succinimidyl ester functional group **(d)**, efficiently modifies lysines in aqueous buffers.

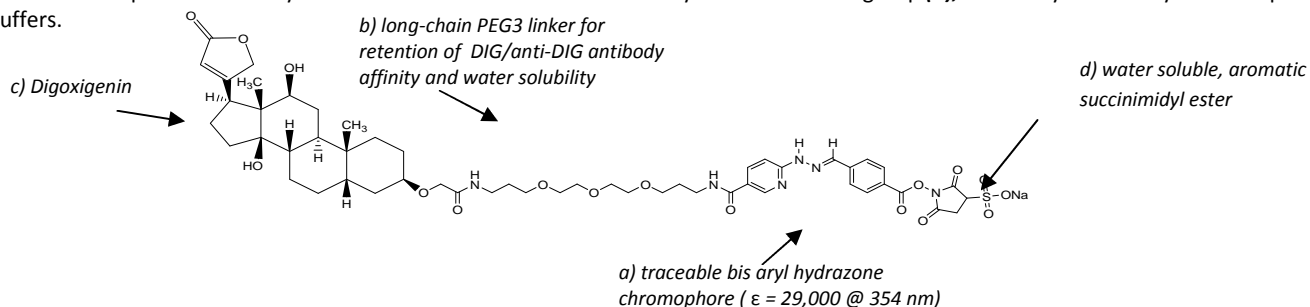


Figure 1. Molecular structure of Sulfo ChromaLink Digoxigenin (water soluble).

Labeling of proteins with Sulfo ChromaLink DIG is the only available method to trace and quantitate DIG incorporated onto a protein. With this reagent, DIG 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 DIG. Spectral analysis illustrates how easy it is to visualize, confirm, and quantify biotin incorporation.

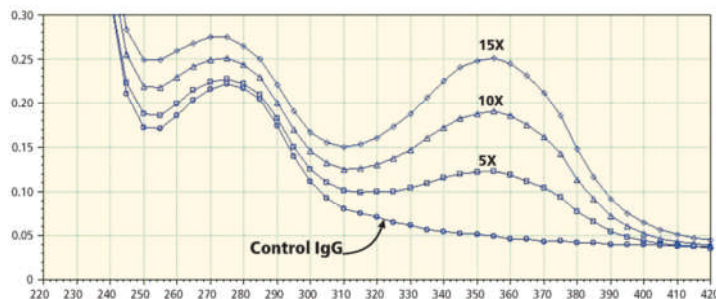


Figure 2. Superimposed spectra of bovine IgG labeled with Sulfo ChromaLink DIG. Various DIG-to-protein mole equivalents (5X, 10X, and 15X) were used. Note the traceable UV signature @ 354 nm, indicating incorporation of DIG. All spectra were scanned on a Molecular Dynamics SpectraMax Plus™ UV-VIS plate 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. No. S-4004-025)
 Modification Buffer (Cat. No. S-4003-005)
 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 benchtop microcentrifuge
 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 7.4). If needed, refer to the [desalting protocol](#).

Notes:

- Buffer exchange removes all free amine-containing contaminants (*e.g.*, tris, glycine) from the protein solution before modification.
- Do not use PBS. High-level buffering capacity, *i.e.*, 100 mM phosphate, is necessary for successful modification.
- For desalting proteins Solulink recommends Zeba Desalt Spin columns (Pierce Cat. No. 89882) - also available through Solulink.

B. Determine the concentration of the protein

- Determine the concentration of the protein to be modified using a [Bradford assay](#) (BioRad, Cat. No. 500-0006) or the [BCA assay](#) (Thermo Scientific, Cat. No. 23223). Alternatively, the A280 can be used if the protein extinction coefficient is known (E1%).
- Bring the concentration to 1–5 mg/mL in Modification Buffer pH 7.4.

C. Prepare a Sulfo ChromaLink DIG/buffer stock solution

- Prepare a stock solution of Sulfo ChromaLink DIG in DMF by dissolving 0.5 mg ChromaLink DIG in 50 μ L anhydrous DMF (provided).

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

D. DIG labeling the protein

- With the aid of the [Protein Modification with an NHS Ester Calculator](#), add 10–20 molar equivalents of Sulfo ChromaLink Digoxigenin stock solution to the protein solution.
 - Note:** Be sure to use the correct values for Sulfo Chromalink Digoxigenin in the **Reagent Information** section of the calculator.
- Allow reaction to incubate at room temperature for 90 minutes.
- After the reaction is complete, quench the reaction by addition of 1/10th volume of 1M Tris (pH 8.9) (not provided).
- Centrifuge the quenched reaction mixture tube @1,500 x g for 30 seconds. After centrifugation, set the tube aside.

E. Desalting procedure

- Desalt/buffer exchange the biotinylated protein into your buffer of choice as directed in part A. If needed, refer to the [Desalting Protocol](#).

F. Determine the concentration of the protein

- Determine the concentration of the DIG-labeled protein using the [BCA assay](#) (Thermo Scientific, Cat. No. 23223).

G. Quantifying degree of DIG modification (MSR)

- Take a UV spectrum of the DIG-labeled protein and use the [E1% Digoxigenin MSR Calculator](#). For optimal labeling, the DIG MSR should be between 2–5, depending of the size of the protein.

Troubleshooting

Problem	Possible Cause	Solution
Protein was not DIG 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 DIG was hydrolyzed	The reagent was left for more than 1 hour in aqueous solution	Use the ChromaLink stock solution <u>immediately</u> 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 DIG-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 labeling reaction is complete, addition of 1 M Tris (pH 9.0) can sometimes be used to resuspend the DIG- 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 DIG stock solution used to label the protein

Stability

The stability of Sulfo ChromaLink DIG-modified proteins varies, and is usually dependent on the stability of the protein itself.

T: 858.625.0670

www.solulink.com

858.625.0770 :F

Related Solulink Products

S-4025 Zeba desalting columns
S-4000 Modification Buffer

B-9014-009K
M-1003

ChromaLink Digoxigenin One-Shot Labeling Kit
MagnaLink Streptavidin Magnetic Beads

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