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QuánťiLink™ Biotin *Sulfo* NHS Ester Labeling Kit

Product No. 1210K

Introduction

QuántiLink™ Biotin *Sulfo* NHS Ester Labeling Kit provides sufficient reagents for 3 labeling reactions each containing 50-500 µg of antibody or other lysine-containing protein in a 100 µL reaction volume. This convenient UV traceable biotin labeling reagent permits rapid quantification of incorporated biotin without the need for a HABA biotin assay.

QuántiLink™ Biotin *Sulfo* NHS Ester is advanced, water-soluble, amine-reactive labeling reagent with built-in signal quantification capability. It was carefully engineered to provide researchers with maximum control and reproducibility over the biotin labeling process while preserving maximum binding affinity to streptavidin. QuántiLink™ Biotinylation reagents contain benzophenone chromophore (ε_{350} = 19,500 $M^{-1}cm^{-1}$) that enables direct and nondestructive quantification of total incorporated biotin by means of spectroscopic A_{280}/A_{350} measurement of a modified protein.

Kit Contents

Component	Amount
QuántiLink™ Biotin-*Sulfo*-NHS Ester Anhydrous	3 x 1 mg
DMSO	1 mL
BupH Saline Buffer (PBS) Pack	1 pack
Zeba™ Spin Columns	6 x 0.5 mL

Materials Required but Not Provided

- UV-VIS Spectrophotometer
- Pipettes and tips (P-10 P-100, P-1000)
- 1.5 mL microfuge tubes
- 6 N NaOH
- Ultrapure water (e.g. 18 MΩ-cm)
- 1L beaker with magnetic stir bar
- Semi-micro quartz cuvette (50-100µL)

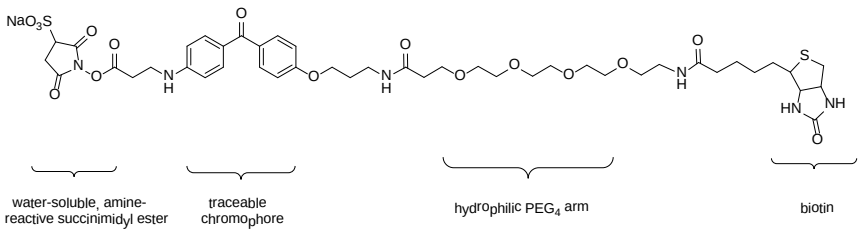

Figure 1. Chemical structure of QuántĭLink™ Biotin *Sulfo* NHS Ester

Protein Requirements

- The protein to be biotinylated must be highly purified and its molecular weight known (e.g. 20-200 kDa).
- The protein must have available primary amines (e.g. N-terminus or lysine amino acid residues).
- Proteins must be free of exogenous primary amines (e.g. glycine or Tris) prior to labeling with NHS esters, if present these compounds must be removed with desalting spin columns.

Important Information

- After reconstitution of QuántĭLink™ Biotin *Sulfo* NHS Ester in DMSO or aqueous buffer, use it immediately. NHS esters readily hydrolyze and become non-reactive. Use only freshly prepared reagent and discard any unused reconstituted reagent.
- For maximum reproducibility, it is preferable to buffer exchange proteins into PBS Buffer (pH 7.5) with the spin columns provided prior to biotinylation. This simple procedure guarantees maximum consistency during the labeling reaction.

Preparation of BupH Buffer (pH 7.5)

- Dissolve the dry-blend BupH buffer pack provided into 500 mL ultrapure water. Adjust the pH of the solution to 7.5 + 0.05 by drop wise addition of 6N NaOH. Adjust the final volume to 500 mL with ultrapure water. For long-term storage sterile-filter the solution. Do not add sodium azide or Proclin preservatives as these reagents interfere with protein A280 measurements.

Protein Preparation

- If the lyophilized protein (50-500 µg) to be biotinylated is pure and free of exogenous amines, resuspend in 100 µL BupH buffer (pH 7.5) to obtain a 0.5-5 mg/mL solution. Proceed to Biotin Labeling Reaction.
- If the lyophilized protein is known to contain exogenous amines (e.g. Tris, glycine) resuspend in 100 µL BupH buffer (pH 7.5) then proceed with buffer exchange using spin columns.

- If the protein to be biotinylated (50-500 µg) is already in 100 µL buffer solution (e.g. 0.5-5 mg/mL PBS), proceed to buffer exchange into BupH buffer (pH 7.5) with spin columns.

Equilibration of Spin Column into BupH (pH 7.5)

1.1 Twist off the column's bottom closure and loosen the red cap. Place each column into a clean 1.5 mL microfuge tube.

1.2 Centrifuge column at 1,500 x g for 2 minutes to remove storage solution. Place a pen mark on the side of the column where the compacted resin is slanted upward. Place column in centrifuge with the mark facing away from the center of the rotor in all subsequent centrifugation steps.
Note-resin will appear white in color and compacted after centrifugation.

1.3 Add 0.3 mL BupH buffer (pH 7.5) to the top of each spin column, replace the cap and loosen.

1.4 Centrifuge at 1500 x g for 2 minutes to remove buffer.

1.5 Repeat steps 3 and 4 two additional times, discarding buffer from the collection tube after each spin.

1.6 Transfer equilibrated spin column (resin appears white and dry) into a clean 1.5 mL microfuge tube and immediately proceed with buffer exchange of protein.

Buffer Exchange of Protein

2.1 Buffer exchange protein into BupH (pH 7.5) equilibrated spin column by slowly applying 100 µL protein solution to the center of the equilibrated resin bed.

2.2 Centrifuge at 1,500 x g for 2 minutes. Retain the eluate at bottom of 1.5 mL collection tube. Discard the used spin column.

2.3 Protein is now buffer exchanged.

Biotin Labeling Reaction

Note: Easy to use **Protein Labeling Calculator** is available for this product at www.clickchemistrytools.com/product/qlink-biotin-sulfo-nhs-ester/

3.1 Select desired excess QuántïLink™ Biotin *Sulfo* NHS Ester to use during the labeling reaction (e.g. 10-fold excess).

3.2 Immediately before use prepare a 20 mM stock solution of QuántïLink™ Biotin *Sulfo* NHS ester by dissolving 1 mg vial in 49 µL of aqueous buffer or water-missible solvent (e.g. DMSO). Vortex for 1-2 minutes to mix well.

3.3 Add required volume of stock solution of QuántïLink™ Biotin *Sulfo* NHS ester to protein solution, pipette the mixture up and down several times to mix.

3.4 Allow labeling reaction to proceed for 1 hour at room temperature.

Removal of Excess Biotin Reagent

4.1 Prepare a buffer exchange spin column as described in **Equilibration of Spin Column into BupH (pH 7.5).**

4.2 Buffer exchange biotin labeled protein as described in **Buffer Exchange of Protein**, use a buffer of choice.

4.3 Determine degree of labeling (DOL) and conjugate protein concentration (mg/mL)

Degree of Labeling (DOL) and Conjugate Protein Concentration (mg/mL)

5.1 Measure conjugate's absorbance at 280 nm and 350 nm in a semi-micro quartz cuvette. **Note-** concentrated protein solutions (e.g. 5 mg/mL) will require dilution (e.g. 1:20) of a small aliquot prior to measurement to achieve desired range (e.g. 0.1 to 1.5 AU). A micro-volume spectrophotometer can be used on small aliquots (1-2 µL) without dilution (e.g. Nanodrop®).

5.2 Calculate the degree of labeling (DOL) and protein concentration with the calculations found in Appendix B, Part I or using **Degree Labeling Calculator** available on this product webpage.

Troubleshooting

Problem	Possible Cause	Solution
Poor or lower than expected biotinylation of proteins	Incorrect protein concentration and/or possible contaminants in protein sample.	Buffer exchange protein into reaction buffer using spin columns and confirm concentration of protein prior to labeling.
	NHS-ester hydrolyzed	Store QuántïLink™ Biotin Sulfo NHS reagent at -20C. Allow product to equilibrate to room temperature before opening.
		Avoid buffers that contain primary amines such as Tris and glycine. Buffer exchange proteins before labeling whenever possible.
	Protein has few or no lysine residues	Check the primary structure of the protein for the presence of lysine residues using the NCBI protein database

	Low A_{350} absorbance of the biotinylated conjugate	Check spectrophotometer lamp for proper functioning
Low conjugate yield	Protein may have aggregated/precipitated during biotinylation	Lower the amount of labeling reagent during the labeling reaction. Use 10% or lower volume DMSO solvent during labeling reaction. Though rare, some proteins become unstable on biotinylation and cannot be labeled.

Appendix A

Part I: Excess QuántĭLink™ Biotin *Sulfo* NHS Reagent to Use in Labeling Reaction

Select the molar excess of QuántĭLink™ Biotin *Sulfo* NHS reagent you wish to use in the labeling reaction. Refer to **Table 1** as a reference guide in the selection process. Typical labeling reactions use a 10 to 20-fold molar excess. Over modification of antibodies or other proteins with biotin can affect their function and stability.

Table 1.

Goat IgG (150 kDa)	**Molar Equivalents**		
	5x	10x	20x
(mg/mL)	DOL	DOL	DOL
0.5	1.1	2.1	4.5
1	1.9	3.7	8.7
2	2.5	6.2	9.7
4	3.1	5.2	9.6

BSA (66.4 kDa)	**Molar Equivalents**		
	5x	10x	20x
(mg/mL)	DOL	DOL	DOL
0.5	2.2	3.5	4.9
1	2.3	4.6	6.1
2	2.2	4.9	7.3
4	2.4	4.7	6.7

Appendix B

Part I. Calculate conjugate's DOL (# biotin/protein) and protein concentration (mg/mL) using Equations 1, 2, 3, and 4 below:

$$\boldsymbol{Eq.1} \qquad number\ of\ biotin\ per\ protein = \frac{molarity\ biotin}{molarity\ protein}$$

$$\boldsymbol{Eq.2} \qquad molarity\ of\ biotin = \frac{A_{350}}{\varepsilon_{350}}$$

$$\boldsymbol{Eq.3} \qquad molarity\ of\ protein = \frac{A_{280C}}{\epsilon_{280}}$$

$$\boldsymbol{Eq.4} \qquad mg/mL = \frac{A_{280} - (A_{350} \times 0.4475)}{\left(\frac{E1\%}{10}\right)} \times dilution\ factor$$

A_{350} = *conjugate absorbance at* $350 \pm 5\ nm$
ϵ_{350} = *molar extinction coefficient QuántïLink Biotin* = $19{,}474\ \mathrm{M^{-1}\,cm^{-1}}$
A_{280} = *conjugate absorbance at* $280\ nm$
A_{280C} = *corrected conjugate absorbance at* $280\ nm = A_{280} - (A_{350} \times 0.4475)$
ϵ_{280} = *molar extinction coefficient protein* $(\mathrm{M^{-1}cm^{-1}}) = \frac{MW_p\ x\ E1\%}{10}$

Example 1: Determine DOL and Conjugate Protein Concentration for the Following Labeling Reaction

A Goat IgG antibody 0.1 mL at 1 mg/mL was labeled with 20-fold molar excess QuántïLink™ Biotin reagent. The (undiluted) conjugate's A280 and A350 were 1.48 and 0.922, respectively. Goat IgG E1% = 13.6 (i.e. 204,000 $\mathrm{M^{-1}\,cm^{-1}}$)

Calculate DOL (# biotin/protein) as follows:

By Equation 2 *molarity of biotin =* $\frac{0.922}{19{,}474\ \mathrm{M^{-1}\,cm^{-1}}}$ *= 47.35 µM*

By Equation 3 *molarity of IgG =* $\frac{1.48 - (0.922 \times 0.4475)}{204{,}000\ \mathrm{M^{-1}\,cm^{-1}}}$ *= 5.23 µM*

By Equation 1 *Number of biotin per IgG =* $\frac{47.35\ \mu M}{5.23\ \mu M}$ *= 9.05*

Calculate conjugate protein concentration (mg/mL)

By Equation 4 *mg/mL =* $\frac{1.48 - (0.922 \times 0.4475)}{1.36} \times 1 =$ *0.78 mg/mL*

Appendix C

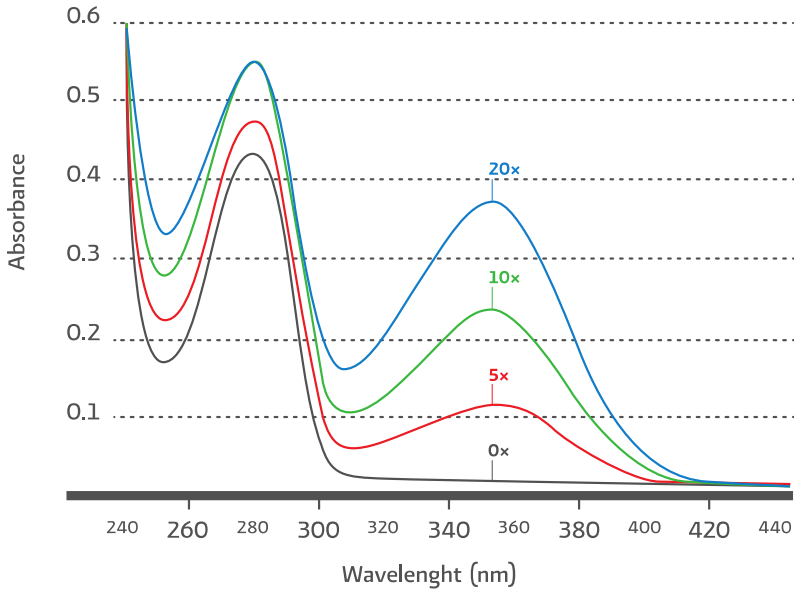

Figure 1. Superimposed absorbance spectra of QuántĭLink™ Biotin labeled Goat IgG at the indicated mole – equivalent excess of labeling reagent over antibody (0×, 5×, 10×, 20×).