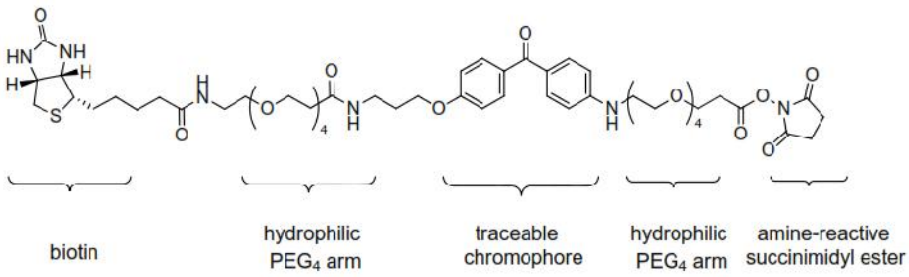
Biotin-NHS ester with Biotrace™ Technical Information and Protocol

Product No.: B-220

Product Name: Biotin-NHS ester with Biotrace™

Chemical Structure:



Chemical Composition: $C_{52}H_{76}N_6O_{17}S$

Molecular Weight: 1089.25 g/mol

Solubility: DMSO, DMF, THF, DCM

Storage: Store at -20°C. (Product shipped at ambient temperature.)

Introduction

Biotin-NHS ester with Biotrace™ is an advanced, 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.

Biotrace™ Biotinylation reagents contain benzophenone chromophore (ε_{350} = 19,500 $M^{-1}cm^{-1}$) that enables direct and nondestructive quantification of total incorporated biotinby means of spectroscopic A_{280}/A_{350} measurement of a modified protein. The UV-traceable chromophore is surrounded by two hydrophilic PEG4 spacers to enhance aqueous solubility and minimize aggregation of biotinylated proteins while preserving maximum binding affinity to streptavidin.

Biotin-NHS ester with Biotrace™ is guaranteed to increase labeling reproducibility and yield for maximum assay robustness.

Product 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 Considerations

- After reconstitution of Biotin-NHS ester with Biotrace™ reagent in DMSO, 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 BupH Buffer (pH 7.5) with the spin columns provided prior to biotinylation. This simple procedure guarantees maximum consistency during the labeling reaction.

Protein Preparation

1. 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.

2. 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.

3. 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.

Biotin Labeling Reaction

1. Select desired excess Biotin-NHS ester with Biotrace™ to use during the labeling reaction (e.g. 10-fold excess).

2. Immediately before use prepare a 100 mM stock solution of Biotin-NHS ester with Biotrace™ by dissolving reagent in anhydrous DMSO. Vortex for 1-2 minutes to mix well.

3. Add required volume of stock solution of Biotin-NHS ester with Biotrace™ to protein solution, pipette the mixture up and down several times to mix.

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

5. Remove excess of Biotin-NHS ester with Biotrace™ reagent with an appropriate desalting column.

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

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®).
2. Calculate the degree of labeling (DOL) and protein concentration with the calculations found in Appendix B, Part I.

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 Biotin-NHS ester with Biotrace™ 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.

Excess Biotin-NHS ester with Biotrace™ Reagent to Use in Labeling Reaction

Select the molar excess of Biotin-NHS ester with Biotrace™ 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.

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

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

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

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

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

A_{350} = *conjugate absorbance at* 350 ± 5 *nm*
ϵ_{350} = *molar extinction coefficient* Biotrace™ *Biotin* = 19,474 $M^{-1}cm^{-1}$
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* ($M^{-1}cm^{-1}$) = $\frac{MW_p\ x\ E1\%}{10}$

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 a 20-fold molar excess Biotrace™ 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 $M^{-1}\ cm^{-1}$)

Calculate DOL (# biotin/protein) as follows:

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

By Equation 3 *molarity of IgG* = $\frac{1.48 - (0.922 \times 0.4475)}{204{,}000\ M^{-1}\ cm^{-1}}$ = *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* = $\frac{1.48 - (0.922 \times 0.4475)}{1.36}$ x 1 = *0.78 mg/mL*

Appendix C.

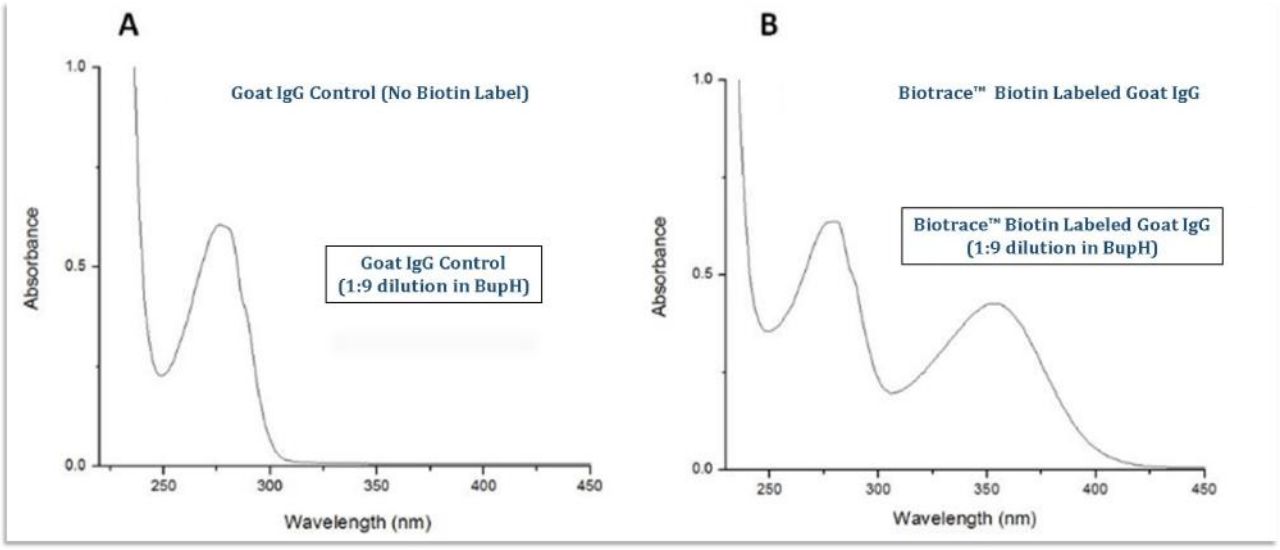


Figure 2. UV scan (230-450 nm) of Goat IgG (unlabeled control) (A), and Biotrace™ Biotin labeled Goat IgG (B). A small aliquot was diluted into BupH (1:9) from a 4 mg/ml stock prior to the scan. The degree of labeling was determined to be 9.6 biotins/ IgG.

For research use only