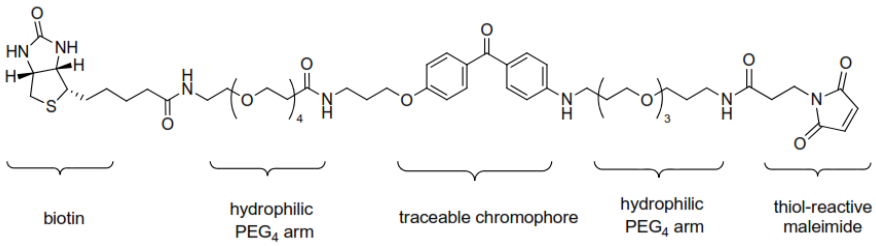
Biotin-SH with Biotrace™

Product No.: B-420

Product Name: Biotin-SH with Biotrace™

Chemical Structure:



Chemical Composition: $C_{54}H_{79}N_7O_{15}S$

Molecular Weight: 1098.31 g/mol

Solubility: DMSO, DMF, THF, DCM

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

Introduction

Biotin-SH with Biotrace™ is an advanced 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 biotin by 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.

The maleimide functional group targets reduced thiols (-SH) while avoiding modification of susceptible lysine residues. Biotin-SH 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 thiols (e.g. cysteines). If absent, sulfhydryls can be added to biomolecules using N-succinimidyl-S-acetylthioacetate (e.g. SATA available from ThermoScientific).
- Proteins must be free of reducing agents (e.g. β-ME, DTT or TCEP) prior to labeling with maleimide esters, if present these compounds must be removed.

Important Considerations

- After reconstitution of Biotin-SH with Biotrace™ reagent in DMSO, use it immediately. Although the maleimide group is more stable than other functional groups, it will hydrolyze to form a nonreactive maleimic acid. Maintain unused stock solution under moisture-free condition (e.g., capped under an inert gas such as argon or nitrogen) at 4°C. Equilibrate reagent vial at room temperature before opening to avoid moisture condensation inside the container.

- The amount of biotin incorporated during the labeling reaction depends primarily on the number and availability of reduced thiols. Generally, a 10- to 20-fold molar excess of reagent is sufficient for most proteins. Over modification of the protein with biotin can affect both function and aqueous stability.

Additional Materials Required

- Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
- Conjugation buffer: Phosphate-buffered saline pH 6.5 or 20mM MOPS, 100mM NaCl pH 6.5.
- Modification buffer: Phosphate-buffered saline (PBS) at pH 7.5.
- Quenching buffer: 1M Tris-HCL, pH 8.0 (optional)
- Desalting Columns (spin or gravity-flow)
- TCEP-HCL and 500mM EDTA (pH 8.0)

Protein Preparation

1. If the lyophilized protein to be biotinylated is pure and free of exogenous thiols (e.g. DTT or β-ME), resuspend in reaction buffer to obtain a 0.2-2 mg/mL solution, proceed to Protein Reduction.

2. If the lyophilized protein is known to contain exogenous thiols (e.g. DTT, β-ME), resuspend in reaction buffer then buffer exchange with spin column.

3. If the purified protein to be biotinylated (100-1000 µg) is already in a suitable thiol-free buffer (e.g. MOPS, Tris-HCL, or PBS) at a concentration range from 0.2-2 mg/mL, proceed to Protein Reduction.

Reduction of Protein (1-3 mg/mL)

1. Prior to conjugation, proteins containing disulfide bonds or free cysteines must be reduced with TCEP (or DTT) to insure proper labeling.

2. Prepare a stock solution of TCEP-HCL (105mM) in ultrapure water, vortex to dissolve crystals completely.

3. Add a volume of 500mM EDTA to protein 1 solution (1-3 mg/mL) to obtain a 1mM final concentration, pipette the mixture up and down several times to mix.

4. Add a volume of TCEP solution (105mM) to protein 1 solution (1-3 mg/mL in Conjugation buffer) to obtain a final concentration of 5mM TCEP, pipette up and down several times to mix.

5. Allow the reaction to incubate for 30 minutes.

6. Buffer exchange TCEP reduced protein 1 into reaction buffer containing 1mM EDTA using an appropriately equilibrated desalting column, pipette up and down several times to mix.

Biotin Labeling Reaction

1. Select desired excess of Biotin-SH with Biotrace™ to use during the labeling reaction (e.g. 20-fold excess).

2. Immediately before use prepare a 100mM stock solution of Biotin-SH 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-SH 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-SH 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 quartz cuvette.

2. Note- concentrated protein solutions (e.g. 2 mg/mL) will require dilution of an small aliquot prior to absorbance measurements, while very dilute solutions (e.g. < 0.2 mg/mL) may need 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®).

3. 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 BupH buffer (pH 6.5) using spin columns provided and confirm concentration of protein prior to labeling.
	Maleimide-ester hydrolyzed	Store Biotin-SH with Biotrace™ reagent at -20C. Allow product to equilibrate to room temperature before opening.
		Avoid buffers that may contain free thiols (β-ME or DTT). Buffer exchange proteins before labeling whenever possible.
	Protein has few or no thiol residues	Check primary structure of protein for the presence of cysteine residues on NCBI protein database. Some proteins may require modification with SATA (or similar reagent) to introduce thiol functional groups.
	Low A_{350} absorbance of the biotinylated conjugate	Check spectrophotometer lamp for proper functioning
Low conjugate yield	Protein may have aggregated/precipitated during biotinylation	Use appropriate relative centrifugal force (e.g. 1000 x g) and recommended spin time to buffer exchange protein. Although rare, some proteins become unstable in aqueous solution on biotinylation and cannot be labeled.

Appendix A.

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

Select the molar excess of Biotin-SH with Biotrace™ reagent you wish to use in the labeling reaction. Typical labeling reactions use 5 to 20-fold reagent molar excess depending on initial protein concentration and the number of available thiols. 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
0.2	0.6	1.6	3.5
1	1.9	3.6	6.5
5	3.4	5.5	9.5

Note: Goat IgG possess 32 cysteine residue.

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 $$molarity\ of\ biotin = \frac{A_{350}}{\epsilon_{350}}$$

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 Biotin-SH with Biotrace™* = 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 0.5 mL at 2.0 mg/mL was labeled using a 20-fold molar excess Biotin-SH with Biotrace™ reagent. The conjugate's A_{280} and A_{350} (1:4 dilution) was determined to be 0.6739 and 0.2112, respectively. Goat IgG E1% = 13.6 (i.e. 204,000 $M^{-1}cm^{-1}$).

Calculate DOL (# biotin/protein) as follows:

By Equation 2 $$molarity\ of\ biotin = \frac{0.2112}{19{,}474\ M^{-1}cm^{-1}} = 10.85\ \mu M$$

By Equation 3 $$molarity\ of\ IgG = \frac{0.6739 - (0.2112 \times 0.4475)}{204{,}000\ M^{-1}\,cm^{-1}} = 2.84\ \mu M$$

By Equation 1 $$Number\ of\ biotin\ per\ IgG = \frac{10.85\ \mu M}{2.84\ \mu M} = 3.82$$

Calculate conjugate protein concentration (mg/mL)

By Equation 4 $$mg/mL = \frac{0.6739 - (0.2112 \times 0.4475)}{1.36} \times 4 = 1.7\ mg/mL$$

Appendix C.

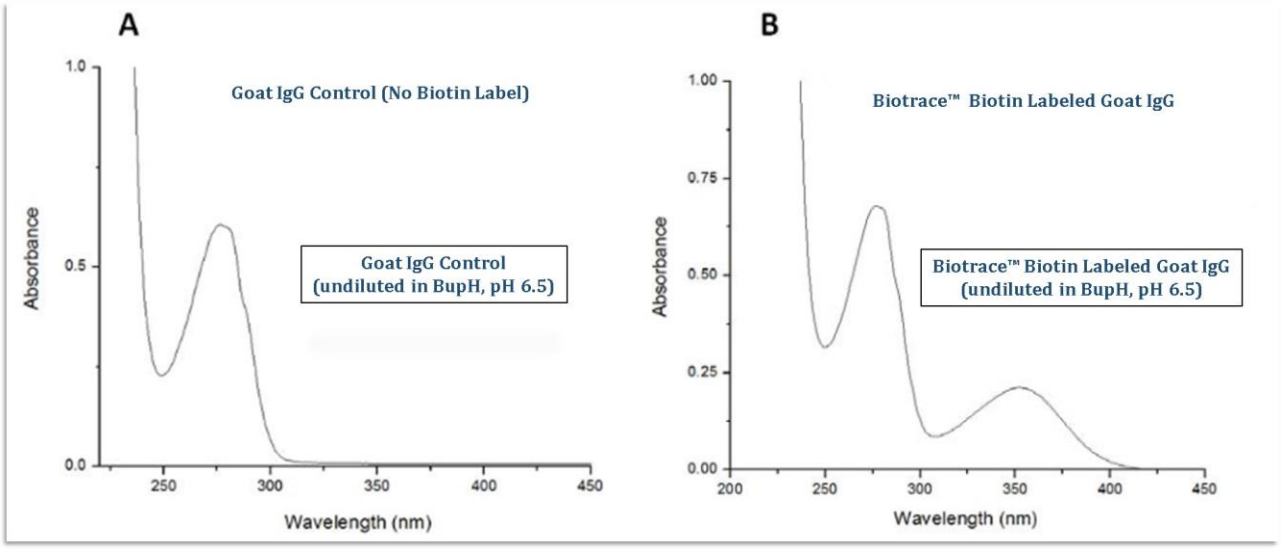


Figure 2. UV scan (230-450 nm) of Goat IgG (unlabeled control) (A), and Biotin-SH with Biotrace™ labeled Goat IgG (B). Samples were scanned (1:4 dilution) at 0.425 mg/mL in BupH (pH 6.5). The degree of labeling was determined to be 3.8 biotins/ IgG.

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