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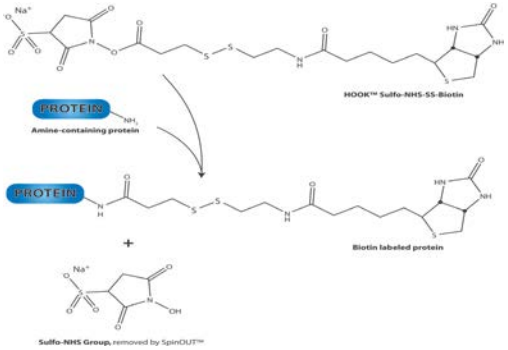
A Geno Technology, Inc. (USA) brand name

HOOK™ Sulfo-NHS-SS-Biotin (Micro)

For the small scale coupling of biotin to protein amine groups

(Cat. # 786-696)

INTRODUCTION



This kit is designed for the conjugation of biotin to protein primary amine groups and is supplied with the biotin reagent, a specific Optimizer Buffer™, for enhanced conjugation, and Spin-*OUT*™ columns for purification of labelled protein.

Biotin, a 244 Dalton molecule, exhibits an extraordinary binding affinity for avidin and streptavidin ($K_a=10^{15}$ M^{-1}). The biotinylated molecules are efficiently probed with avidin or streptavidin conjugated to reporter molecules, such as peroxidases or phosphatases. The use of biotin labeled proteins in ELISA, Western blotting and dot blotting is a popular technique.

The most widely used amine reactive biotinylation reagents are the water insoluble *N*-hydroxysuccinimide (NHS) esters or the water soluble *N*-hydroxysulfosuccinimide (sulfo-NHS) esters. The addition of a charged sulfonate (SO_3^-) on the *N*-hydroxysuccinimide ring of the sulfo-NHS esters results in their solubility in water (~10mM), but not permeable to plasma membranes. The solubility and impermeability to plasma membranes makes them ideal for studying cell surface proteins as they will only react with the protein molecules on the outer surface of plasma membranes. The reactions of the NHS and sulfo-NHS esters with amines are virtually identical leading to the formation of an amide bond and release of NHS or sulfo-NHS.

HOOK™ Sulfo-NHS-SS-Biotin has a disulfide bridge in its spacer arm that allows the biotin moiety to be removed by treatment with DTT, or other reducing agent. This feature is useful when the protein is purified by streptavidin or avidin affinity chromatography as the protein can be readily eluted in a DTT containing buffer.

HOOK™ Sulfo-NHS-SS-Biotin kit is designed for the coupling of 50-250µg protein in 0.2-0.7ml buffer, suitable for 8-10 couplings.

ITEM(S) SUPPLIED (Cat. # 786-696)

Description	Size
OneQuant™ HOOK™ Sulfo-NHS-SS-Biotin Agent	8 x 1mg
Optimizer Buffer™ I [5X]	2 x 25ml
Spin-OUT™ GT-600, 3ml	10 columns

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components at 4˚C, except the biotin reagent should be stored at -20˚C.

SPECIFICATIONS

Molecular weight: 606.69
Spacer Arm (Å): 24.3
Membrane Permeable: No
Water Soluble: Yes
Reaction pH: 7-9

PRECAUTIONS

1. Dissolve the HOOK™ Sulfo-NHS-SS-Biotin immediately prior to use as the NHS-ester will hydrolyze and become inactive. Do not prepare stock solutions.
2. Avoid using primary amine containing buffers, such as Tris and Glycine, as these will compete with the reaction.

ITEMS NEEDED BUT NOT SUPPLIED

15ml collection tubes

PREPARATION BEFORE USE

Dilute and prepare 1X Optimizer Buffer™ (1ml 5X Optimizer Buffer™ per 4ml de-ionized water).

A. PROTEIN SAMPLE PREPARATION

1. Dissolve 50-250µg protein in 0.2-0.5ml 1X Optimizer Buffer™ I.
2. If your protein is in an amine–free buffer at a pH of 7.2-8.0 then proceed to the next section.
3. For protein in Tris or other amine containing buffers a buffer exchange must be performed. The buffer exchange can be done by dialysis against Optimizer Buffer™ I, we recommend using our Tube-O-DIALYZER™ micro dialysis devices that ensure no loss of precious protein (See Appendix 1). Or one of the supplied Spin-*OUT*™ columns can be used for buffer exchange

as described in Section D. Please note this kit is designed for 10 reactions and the Spin-*OUT*™ columns are for purification of the biotin labeled protein, using a column for buffer exchange will reduce the number of reactions that can be performed. Additional columns can be ordered at www.GBiosciences.com.

B. CALCULATION OF QUANTITY OF BIOTIN AGENT NEEDED FOR CONJUGATION

To achieve approximately ~3 biotin groups per antibody molecule, we recommend using a 50 molar excess of biotin to dilute antibody/ protein solutions. The extent of biotin labeling for other proteins is dependent on the distribution of amine groups and size of the protein, therefore the molar ratio can be adjusted to suit your needs.

1. Millimoles of HOOK™ Sulfo-NHS-SS-Biotin to be added for a 50 mole excess:

$$\text{Protein Sample Volume (ml)} \times \frac{\text{Protein Sample Concentration (mg/ml)}}{\text{Protein Mol. Wt (Da)}} \times 50 = \text{mmol HOOK™ Sulfo-NHS-SS-Biotin}$$

2. µl HOOK™ Sulfo-NHS-SS-Biotin to add:

$$\text{mmol HOOK™ Sulfo-NHS-SS-Biotin} \times 606.69 \times \frac{200}{1} = \text{µl HOOK™ Sulfo-NHS-SS-Biotin solution}$$

606.69= HOOK™ Sulfo-NHS-SS-Biotin molecular weight
200 = µl of water added to 1mg of HOOK™ Sulfo-NHS-SS-Biotin

Example: For 0.2ml of a 0.5mg/ml IgG solution (150,000 Mol. Wt) solution.

$$0.2ml \times \frac{0.25mg/ml}{150{,}000Da} \times 50 = 1.66x10^{-5}\ mmol\ HOOK™\ Sulfo\text{-}NHS\text{-}SS\text{-}Biotin$$

$$1.66x10^{-5}\ mmol\ HOOK™\ Sulfo\text{-}NHS\text{-}SS\text{-}Biotin \times 606.69 \times \frac{200}{1} = 2\mu l\ HOOK™\ Sulfo\text{-}NHS\text{-}SS\text{-}Biotin\ solution$$

C. PREPARATION OF BIOTIN AGENTS

1. Cut a vial of HOOK™ Sulfo-NHS-SS-Biotin from the strip and return remainder to -20°C. Warm the biotin-agent vial to room temperature before opening.
2. Immediately before using, pierce the foil top with a pipette tip and add 200µl deionized water to the HOOK™ Sulfo-NHS-SS-Biotin for a working solution. Mix by pipetting up and down.
   NOTE: *Make fresh each time and do not prepare stock solutions.*

D. BIOTIN CONJUGATION REACTION

1. Add the calculated volume (Section B) of freshly prepared HOOK™ Sulfo-NHS-SS-Biotin to the protein solution from Section A.
2. Incubate the reaction at room temperature for 30-60 minutes or on ice for 2 hours. Longer incubations can be performed, but these may be affected by protein degradation.

E. REMOVAL OF UNCONJUGATED BIOTIN AGENTS

1. Prepare the Spin-*OUT*™ column by removing the top and then bottom caps. Place into a 15ml collection tube.
2. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer. Discard storage buffer and return column to 15ml collection tube.
3. Equilibrate the column with 1ml 1X Optimizer Buffer™ I, by adding slowly to the resin bed. Centrifuge at 1,000g for 2 minutes. Discard flow through and repeat this step a further 2 times.
4. Place the column in to a clean 15ml collection tube and apply the sample directly to the center of the resin bead. Allow the sample to migrate into the resin bed.
5. Centrifuge the column at 1,000g for 2 minutes. The flow through is the purified labeled protein sample.
6. Store biotinylated protein at 4°C in 0.1% sodium azide until ready for use. Store at -20°C for long term storage.

APPENDIX 1: SAMPLE EQUILIBRATION WITH TUBE-O-DIALYZER™ (NOT SUPPLIED)

If protein solution is in an incompatible buffer, dialyze and equilibrate into 1X Optimizer Buffer™ as follows:

1. Pipette your sample directly into the Tube-O-DIALYZER™ tube. For Tube-O-DIALYZER™ Micro use 20-250µl and for Tube-O-DIALYZER™ Medi use 0.2-2.5ml.
   NOTE: Tube-O-Dialyzer™ is available in 1, 4, 8, 15 and 50kDa MWCO. Visit our website for further information.
2. Pipette 3-5ml appropriate 1X Optimizer Buffer™ into a Micro Dialysis Cup or small beaker. If a small magnetic stir bar is available add to the Micro Dialysis Cup, if not add 3-5 glass balls.
3. Screw the dialysis cap on to the Tube-O-DIALYZER™ tube. Invert the Tube-O-DIALYZER™, ensuring the entire sample rests upon the membrane.
   NOTE: *If sample is too viscous, centrifuge the Tube-O-DIALYZER™ in an inverted position (i.e. the dialysis membrane facing downward). Centrifuge for 5 seconds at 500-1,000g.*
4. Keeping the Tube-O-DIALYZER™ in an inverted position, slide the supplied float onto the Tube-O-DIALYZER™ tube. Place the Tube-O-DIALYZER™ in the Micro Dialysis Cup with the Optimizer Buffer™.
5. Ensure that the dialysis membrane contacts the dialysis buffer. If there are large air bubbles trapped underneath the dialysis membrane surface, tilt the tube or squirt buffer to remove the air bubbles. Gently, stir the dialysis buffer with a magnetic stir or place on an orbital shaker. For efficient and complete dialysis we recommend inverting or gently tapping the Tube-O-DIALYZER™ 1-2 times during dialysis to mix the sample. If necessary repeat the centrifugation in step 3.
6. Dialyze at room temperature, or 4°C if required, for 1-2 hours.
7. Repeat the dialysis with 1-2 changes of buffer.
8. After dialysis, remove the Tube-O-DIALYZER™ from the float and immediately spin the Tube-O-DIALYZER™ (in up-right position) for 5-6 seconds at 500-1,000xg.

APPENDIX 2: INSTRUCTIONS FOR PROTEIN ELUTION WITH DTT

1. Prepare a 50mM DTT solution in an appropriate elution buffer and then add 1 bed volume to your streptavidin/avidin column that has the bound and washed biotinylated protein.
2. Incubate the column for 30-60 minutes at room temperature preferably on a rotator. Alternatively, rock back and forth on a rocking platform.
3. Collect the eluted protein.

RELATED PRODUCTS

Download our Protein Labeling & Conjugation and Antibody Production Handbook.

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