G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

HOOK™ IgG Biotinylation (Amine)

Solid Phase Biotinylation & Rapid Purification of IgG Molecules

(Cat. # 786-728)

INTRODUCTION

The HOOK™ IgG Biotinylation (Amine) kit is designed for the efficient biotinylation of IgG molecules by immobilizing the IgG molecules on a solid support. IgG molecules contain a histidine rich region in their F_c region and this feature is used to first immobilize the IgG molecules on a Nickel Chelating Resin support. The immobilized IgG molecule is then incubated with NHS-dPEG$_4$-Biotin solution to biotinylated the free amine groups. The free NHS-dPEG$_4$-Biotin is washed away and the biotinylated IgG is eluted with an imidazole containing buffer. The biotinylation of the IgG on the Nickel Chelating resin results in the addition of 3-5 biotin molecules per IgG molecule.

The HOOK™ IgG Biotinylation (Amine) kit offers an advantage over standard biotinylation reactions as the immobilization to the Nickel Chelating resin allows for the rapid removal of uncoupled biotin and therefore eliminates the need for further dialysis or desalting of the biotinylated antibody.

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 kit is supplied with a Nickel Chelating column that can be regenerated up to 10 times and our single-use aliquots of NHS-dPEG$_4$-Biotin (See Appendix A for structure and coupling reaction). 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.

The advantage of using a PEG (polyethylene glycol) biotinylation reagent is that the long hydrophilic spacer arm conveys its water solubility to the molecule that it is coupled to. This means that antibodies and other proteins labeled with our NHS-dPEG$_4$-Biotin have a reduced occurrence of aggregation compared to non-PEG biotinylation reactions.

HOOK™ IgG Biotinylation (Amine) kit is designed for the coupling of 1-10mg protein in 1ml buffer, suitable for 8 couplings.

ITEM(S) SUPPLIED (Cat. #786-728)

Part. #	Description	Size
145O	OneQuant™ NHS-dPEG$_4$-Biotin Agent	8 x 1mg
059N-B	Nickel Chelating Column	1
001J	JAW™ Phosphate Buffered Saline Pack	1L
021H	His Elution Buffer	100ml

STORAGE CONDITIONS

Shipped at ambient temperature. Store at 4°C, do not freeze.

SPECIFICATIONS FOR NHS-dPEG$_4$-BIOTIN

- Molecular weight: 588.67
- Spacer Arm (Å): 29
- Membrane Permeable: No
- Water Soluble: Yes
- Reaction pH: 7-9

IMPORTANT INFORMATION

- This kit is designed for the biotinylation of purified IgG molecules. For IgG molecules in serum, ascites or cell culture supernatant we recommend purifying with our IgG Purification kits (see Related Products).
- This kit is not suitable for IgM, IgY, F_{ab} or other antibody fragments lacking the histidine rich F_c domain.
- Commercial antibodies can be biotinylated with this kit, however the presence of the protein stabilizer BSA, and to a lesser extent, gelatin will compete for biotinylation and will elute and contaminate the IgG sample. We recommend our Pearl™ Antibody Clean Up kit (Cat. # 786-803) for the removal of the BSA and gelatin protein stabilizers.
- The NHS-ester of the biotin reagent is readily hydrolyzed, therefore reconstitute the NHS-dPEG$_4$-Biotin vials immediately prior to use and discard unused reagent. Do not prepare stock solutions.

ADDITIONAL ITEM(S) REQUIRED

- Collection tubes
- Protein/Peptide solution in an aqueous buffer
- Equilibration Buffer: Any aqueous buffer, pH6.5-8.0

PREPARATION BEFORE USE

1. 1X PBS: Add the entire contents of the JAW™ Phosphate Buffered Saline Pack to 1 liter of distilled water.
2. Antibody Solution: Use IgG that is free from BSA and gelatin protein stabilizers. Dilute 1-10mg IgG in 6ml PBS. If required, large volumes can be used; however the maximum amount of IgG that will bind the column is 10mg.
3. Briefly centrifuge the column at 1,000xg for 1-2 minutes to pack the resin in the bottom of the column.

PROCEDURE

Antibody Immobilization

1. Allow the Nickel Chelating Column and PBS to warm to room temperature.
2. Remove the top, then bottom cap of the resin column and allow the storage buffer to drain out of the column.
3. Equilibrate the resin with 15ml 1X PBS and allow the PBS to drain through the column.
4. Pipette the Antibody Solution onto the center of the column and allow it to flow into the gel.
5. Wash the column with 15ml 1X PBS.

Antibody Biotinylation

1. Pierce the foil of the OneQuant™ NHS-dPEG$_4$-Biotin Agent and pipette in 200µl PBS. Dissolve the biotin reagent but slowly pipetting up and down.
2. Prepare the NHS-dPEG$_4$-Biotin Labeling Solution in a suitable tube by adding the quantities indicated in the table below. Add the PBS first.

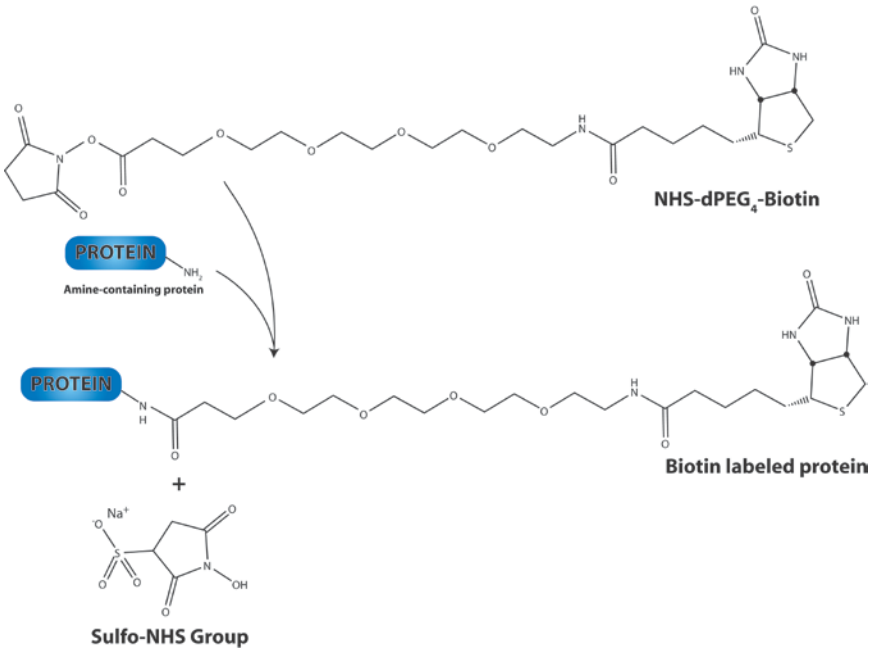
Antibody Amount (mg)	PBS Volume (ml)	Biotin Volume (µl)	Biotin Final Molarity
1-2.5	3	177	0.5
2.6-4.5	3	282	0.8
4.6-5.9	1.5	195	1.1
6-7.5	1	200	1.7
7.6-10	0.68	200	2.5

3. Immediately apply the NHS-dPEG$_4$-Biotin Labeling Solution to the column and allow the solution to flow through the column.
4. Once the flow has stopped cap the bottom and then top of the column, then incubate for 30 minutes at room temperature.
5. Following the 30 minute incubation remove the top then bottom cap and wash the resin with 15ml 1X PBS.

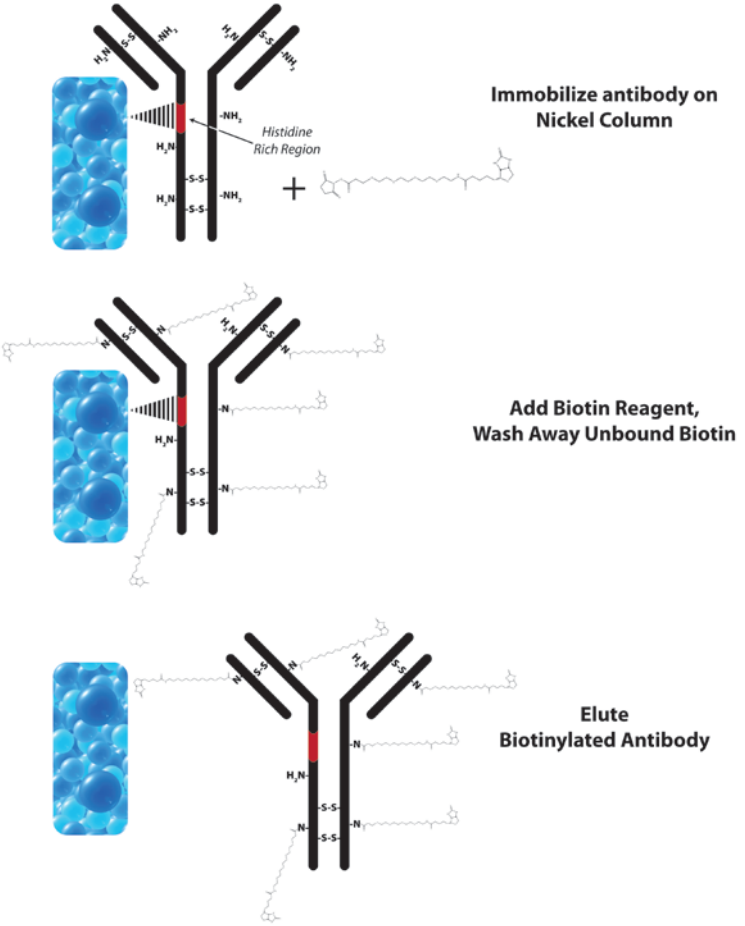
Antibody Elution

1. Transfer the column to a clean collection tube and add 3ml elution buffer to the column.
2. Collect the biotinylated antibody. The antibody can be stored in the elution buffer at 4°C, for long term storage store at -20°C.
3. Regenerate the Nickel Chelating Column with the addition of 3ml Elution Buffer and then wash with 12ml 20% ethanol containing 0.02% sodium azide. Add the bottom cap and add 3ml 20% ethanol containing 0.02% sodium azide. Seal with the top cap and store at 4°C. The column can be used up to 10 times if washed and stored correctly.

APPENDIX 1: NHS-dPEG$_4$-BIOTIN STRUCTURE AND COUPLING SCHEME



APPENDIX 2: HOOK™ IGG BIOTINYLATION (AMINE) SCHEME



RELATED PRODUCTS

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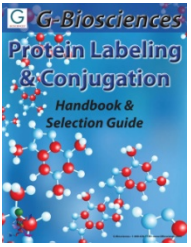

http://info.gbiosciences.com/complete-protein-labeling-conjugation-handbook

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