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

A Geno Technology, Inc. (USA) brand name

HOOK™ IgG Biotinylation (Sulfhydryl)

Solid Phase Biotinylation & Rapid Purification Of IgG Molecules

(Cat. #786-729)

INTRODUCTION

The HOOK™ IgG Biotinylation (Sulfhydryl) 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 disulfide bonds of the immobilized IgG molecule are then reduced with the supplied Protein-S-S-Reductant™, with excess reductant being simply washed away. The reduced immobilized IgG molecule is then incubated with PEG_2-Iodoacetyl-Biotin solution to biotinylated the free sulfhydryl groups. The free PEG_2-Iodoacetyl-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 2-4 biotin molecules per IgG molecule.

The HOOK™ IgG Biotinylation (Sulfhydryl) 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 PEG_2-Iodoacetyl-Biotin (See Appendix A for structure). PEG_2-Iodoacetyl-Biotin is a sulfhydryl reactive biotinylation reagent that react with thiol groups at pH7.5-8.5 and forms stable thioether bonds. PEG_2-Iodoacetyl-Biotin is water soluble, due to its polyethylene glycol (PEG) spacer arm. For specific reaction with sulfhydryls, limit the reaction to pH 7.5-8.5 and the molar ratio of iodoacetyl-biotin to protein such that the concentration of biotin is only slightly higher than the sulfhydryl concentration. Iodoacetyl reaction should be performed in dark to limit the formation of free iodine, which has the potential to react with tyrosine, tryptophan, and histidine residues.

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 PEG_2-Iodoacetyl-Biotin have a reduced occurrence of aggregation compared to non-PEG biotinylation reactions. HOOK™ IgG Biotinylation (Sulfhydryl) kit is designed for the coupling of 1-10mg protein in 1ml buffer, suitable for 8 couplings.

ITEM(S) SUPPLIED (Cat. # 786-729)

Part. #	Description	Size
249H-C	OneQuant™ PEG_2-Iodoacetyl-Biotin	8 x 2mg
059N-B	Nickel Chelating Column	1
512P-B	Protein-S-S-Reductant™	1ml
013J	JAW™ Tris Buffered Saline Pack	1L
021H	His Elution Buffer	100ml

STORAGE CONDITIONS

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

SPECIFICATIONS FOR PEG_2-IODOACETYL-BIOTIN

- **Synonym:** PEO-Iodoacetyl-Biotin
- **Molecular Weight:** 542.43
- **Spacer Arm (Å):** 24.7
- **Reactive Group:** Iodoacetyl
- **Cleavage/Reversible:** NO
- **Water Soluble:** YES
- **Reaction pH:** 7.5-8.5

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 iodoacetyl reactive group of the biotin reagent is readily hydrolyzed, therefore reconstitute the PEG_2-Iodoacetyl-Biotin vials immediately prior to use and discard unused reagent. Do not prepare stock solutions.

ADDITIONAL ITEMS REQUIRED

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

PREPARATION BEFORE USE

1. 1X TBS: Add the entire contents of the JAW™ Tris 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 TBS. 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 TBS 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 TBS and allow the TBS 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 TBS.

Antibody Reduction

1. Prepare the Protein-S-S-Reductant™ Solution in a suitable tube by adding the indicated amount of Protein-S-S-Reductant™ to 6ml 1 X TBS.

Antibody Amount (mg)	Protein-S-S-Reductant™ Volume (µl)
1	3
2	5.5
3	8.5
4	11
5	13
6	16
7	19
8	22
9	24
10	27

2. Immediately apply the Protein-S-S-Reductant™ Solution to the column and allow the solution to flow through the column.
3. Once the flow has stopped cap the bottom and then top of the column, then incubate for 30 minutes at room temperature.

4. Following the 30 minute incubation remove the top then bottom cap and wash the resin with 15ml 1X TBS.

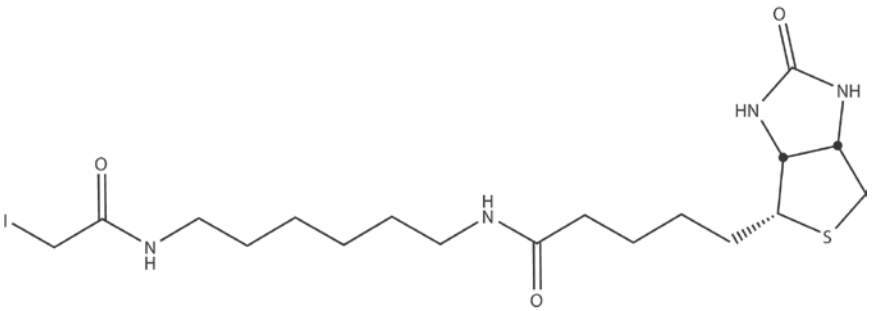
Antibody Biotinylation

1. Pierce the foil of the OneQuant™ PEG_2-Iodoacetyl-Biotin Agent and pipette in 200µl TBS. Dissolve the biotin reagent but slowly pipetting up and down.
2. Add the entire 200µl PEG_2-Iodoacetyl-Biotin solution to a suitable tube containing 2.5ml 1X TBS
3. Immediately apply the PEG_2-Iodoacetyl-Biotin 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 TBS.

Antibody Elution

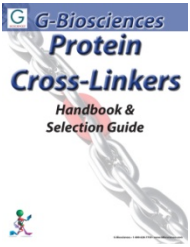
1. Transfer the column to a clean collection tube and add 3ml elution buffer to the column.
2. Collect the biotinylated antibody. The antibody can be stored in the elution buffer at 4°C, for long term storage store at -20°C.
3. Regenerate the Nickel Chelating Column with the addition of 3ml Elution Buffer 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 A: PEG_2-IODOACETYL-BIOTIN STRUCTURE



RELATED PRODUCTS

Download our Protein Cross-Linkers Handbook.



http://info2.gbiosciences.com/complete-protein-cross-linkers-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 5/19/2015 CMH

www.GBiosciences.com