

Biotinylation is a simple and gentle labeling procedure and is particularly suited for labeling monoclonal antibodies or affinity purified polyclonal antibodies. Biotin-N-hydroxysuccinimide esters (NHS-Biotin) will biotinylate primary amine groups of both proteins and nucleic acids. The extended spacer arm present in the NHS-LC-Biotin molecule enhances the formation and stability of avidin/biotin complexes due to a reduction in steric hindrance associated with avidin binding several biotinylated molecules.

The Long Chain NHS-Biotinylation Kit provides sufficient reagents to label 50 mg of antibody.

#### **CONTENTS OF KIT:**

1. Long Chain NHS-Biotin - 5 mg
2. Carbonate Buffer Concentrate - 100 ml
3. PBS Buffer Concentrate - 50 ml
4. Sephadex G25 column - 1 each

#### **STORAGE OF KIT:**

This kit can be stored refrigerated for short-term storage. The buffers and the G25 column can be stored at room temperature, but once the column has been used it is best to store it in the refrigerator in 1X PBS. The powdered biotin can be stored refrigerated for short term but should be kept frozen and desiccated for long term storage. Always allow the biotin to come to room temperature before opening the vial.

**PRODUCTS FOR RESEARCH USE ONLY**

**AMERICAN QUALEX Manufactures "QUALITY & EXCELLENCE"**

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## BIOTINYLATION OF ANTIBODIES

### PROCEDURE:

1. Reconstitute 5 mg Protein (antibody) in 0.55 ml distilled water if lyophilized. Add 0.05 ml Carbonate Buffer Concentrate. Alternatively, protein can be dialyzed against a 1:10 dilution of Carbonate Buffer Concentrate overnight.

#### Sample Calculation:

$$\frac{20 \text{ mmole Biotin}}{1 \text{ mmole Protein}} \times \frac{1 \text{ mmole Protein}}{150,000 \text{ mg Protein}} \times \frac{556.6 \text{ mg Biotin}}{1 \text{ mmole Biotin}} = \frac{0.0742 \text{ mg Biotin}}{\text{mg Protein}}$$

2. Remove NHS-Biotin from refrigerator and allow it to warm to room temperature. Do not open until temperature has stabilized. For best results, store any unused biotin at -20°C, desiccated.
3. Dissolve 0.5 mg Biotin in 0.5 ml distilled water.
4. Add Biotin solution to Protein solution and gently shake or stir for 1 hour at room temperature.
5. Remove reaction mixture and pass over G25 column to remove un-reacted biotin. Maximum sample volume for column is 2.5 ml. Be sure to equilibrate the column in 1X PBS before use. The biotin conjugate will come off of the column first, followed by un-reacted protein, and then finally, un-reacted biotin. (Alternatively, conjugate may be dialyzed against a 1:20 dilution of PBS Buffer Concentrate overnight).
6. Stabilization of biotinylated protein solution may be accomplished by addition of BSA to a final concentration of 1% (w/v). Store at 2 – 8 °C.

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## BIOTINYLATION OF PEPTIDES

Using the procedure recommended above, approximately 120  $\mu$ mole of peptide (assuming 1 available primary amine per molecule) may be labeled with biotin in aqueous solution.

Following is a sample protocol for biotinylating peptides in organic solvents.

### MATERIALS NOT INCLUDED IN KIT:

Imidazole - 5 mg  
DMSO - 1 ml

### PROCEDURE:

1. Dissolve 5 mg of imidazole in 1 ml of DMSO.
2. Add 120  $\mu$ mole of peptide to the imidazole/DMSO solution.
3. Add 5 mg of NHS-biotin to the above mixture with stirring.
4. Stir for 6 hours at room temperature.
5. The biotinylated peptide solution may be desalted on a prepacked gel filtration column with a molecular weight cut-off of 1000. Note: If beginning with dry gel filtration matrix, swell the matrix in DMSO before packing. If the gel filtration matrix is in aqueous buffer, it may be necessary to do a step-wise gradient preparation of the matrix to prevent damage to the support. Wash first with 100% water, then 30% DMSO, followed by a wash with 70% DMSO in water and finally equilibrate with 100% DMSO before applying the sample to the column.

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**K8000  
ANTIBODY BIOTINYLATION KIT**

**Kit Components**

**Kit Lot #: 40199D**

73 - 40197D	NHS LC Biotin	5 mg
74 - 40198D	Carbonate Buffer Concentrate	100 ml
75 - 30385G	Sephadex G 25 Column	1 each
38 - 20739J	PBS Buffer Concentrate	50 ml

**Store at 2 – 8 °C**

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