

EZ-Link[®] NHS-PEG Solid Phase Biotinylation Kit – Pre-packed Column

21440

1473.2

Number	Description
21440	<p>EZ-Link NHS-PEG Solid Phase Biotinylation Kit – Pre-packed Column, contains sufficient material for eight biotinylation reactions each consisting of 1-10mg of IgG</p> <p>Kit Contents:</p> <p>Immobilized Nickel Chelated Column, 1mL Binding capacity: >10mg human IgG</p> <p>NHS-PEG₄-Biotin, No-Weigh[™] Format, 8 × 2mg microtubes Molecular Weight: 588.67 Spacer Arm Length: 29Å</p> <p>BupH[™] Phosphate Buffered Saline Pack, 1 each</p> <p>4M Imidazole Stock Solution, 5mL</p>

Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Table of Contents

Introduction	1
Important Product Information	2
Additional Materials Required.....	2
Material Preparation	2
Procedure for Solid-Phase Biotinylation.....	3
A. Antibody Binding	3
B. Antibody Biotinylation.....	3
C. Antibody Elution	3
Troubleshooting.....	3
Appendix	4
Related Thermo Scientific Products	4
Cited References.....	4

Introduction

The Thermo Scientific EZ-Link NHS-PEG Solid Phase Biotinylation Kit allows for efficient biotinylation of purified IgG class antibodies. This solid-phase biotinylation method uses nickel-chelated agarose to first immobilize purified IgG. The antibody is then biotinylated by adding a solution of NHS-PEG₄-Biotin. Excess biotin is washed from the column, and the antibody is eluted in a buffered imidazole solution. The reaction results in approximately 3-5 biotin molecules per antibody molecule. Although this solid-phase format has been optimized using human IgG, it may be used with other mammalian antibodies. The nickel-chelated agarose binds IgG through a histidine-rich cluster on the Fc region at the junctures of the C_γ2 and C_γ3 domains that is highly conserved among all mammalian IgGs.¹⁻⁴ Purified IgG from sheep, mouse, goat, rat and rabbit will bind to nickel-chelated resin.

This kit includes NHS-PEG₄-Biotin packaged in the convenient pre-measured No-Weigh Microtube Format, eliminating difficulties associated with weighing small quantities of reagent. NHS-PEG₄-Biotin (see Figure 1 in Appendix A for molecular structure) reacts with primary amines, primarily ε-amine groups on available lysine residues. The *N*-hydroxysuccinimide (NHS) ester reacts with amines by nucleophilic attack, forming an amide bond and releasing the NHS. The resulting biotinylated antibody retains biological activity because biotin is a relatively small molecule. An antibody conjugated with several biotin molecules can each bind one molecule of avidin, thereby increasing the sensitivity of many

assays. The bond formation between biotin and avidin is rapid and, once formed, is unaffected by most extremes of pH, organic solvents and other denaturing agents.⁵ The hydrophilic polyethylene glycol (PEG) spacer arm of NHS-PEG₄-Biotin imparts water solubility that is transferred to the biotinylated antibody, thus NHS-PEG₄-Biotin reduces aggregation of labeled antibodies stored in solution.⁶

This solid-phase method is advantageous compared with solution-phase protocols as it facilitates reagent delivery and removal of spent product and there is more control over reaction conditions. Although the time required for completion of the protocol is comparable to solution-phase protocols, antibody immobilization eliminates the need for desalting or dialysis to remove excess biotin, resulting in excellent antibody recovery.

Important Product Information

- Use this kit only with purified IgG. Antibodies in serum or ascites must be purified before using this kit. Do not use this kit for IgM or IgY, Fab, or antibody fragments that do not contain a Fc region, as they do not bind efficiently to the nickel-chelated agarose.
- This protocol has been optimized for 1-10mg of antibody. The antibody preparation must be free of chelating agents such as EDTA and EGTA.
- BSA (bovine serum albumin) is often added to commercial antibody preparations as a stabilizer and is present in molar excess to the antibody. BSA will decrease specific biotinylation because it contains available histidine residues and binds to the nickel-chelated agarose and is then biotinylated and eluted along with the antibody. Remove BSA before using this kit. BSA removal is a fast and simple process; see Appendix B for suggested albumin removal products.

Note: Although gelatin, which often is also added to antibody preparations, will bind to the nickel-chelated agarose, it is present in low amounts (usually ~0.2%) and will not significantly affect yields.

- Use reconstituted No-Weigh NHS-PEG₄-Biotin immediately. The NHS-ester moiety readily hydrolyzes and becomes nonreactive; therefore, solutions cannot be prepared for storage. Discard any unused reconstituted reagent.
- The degree of biotinylation can be determined by performing the HABA assay (Product No. 28005); however, 0.2M imidazole (Elution Buffer) interferes with the HABA assay. Dilute on-column biotinylated IgG 1:1 with PBS before use in the HABA assay to reduce imidazole concentration to 0.1M.
- Protein assays can be used to determine concentration of eluted IgG. When determining concentration of IgG in Elution Buffer, use the Thermo Scientific Coomassie Plus (Bradford) Protein Assay Reagent (Product No. 23236). The Thermo Scientific Pierce BCA Protein Assay cannot be used because imidazole interferes with the assay chemistry.
- When properly washed and stored, the nickel-chelated column can be re-used up to 10 times without significant loss of binding capacity.

Additional Materials Required

- 0.2 µm, 500mL filter sterilization unit
- Test tubes and test tube rack

Material Preparation

Phosphate Buffered Saline (PBS)	Reconstitute contents of the phosphate-buffered saline (PBS) pack with 500mL ultrapure water. Filter-sterilize solution using a 0.2µm filter apparatus and store at 4°C. When stored properly, there is sufficient buffer for eight antibody biotinylation reactions of up to 10mg of IgG each.
Elution Buffer	Prepare 6mL of Elution Buffer by diluting 300µL of the 4M Imidazole Stock Solution with 5.7mL of PBS.
Antibody Binding Solution	Dilute purified IgG to be biotinylated (1-10mg) with PBS to a volume of 6mL. If the antibody concentration is too dilute to make a 6mL binding solution, dilute the antibody 1:1 with PBS and apply the entire volume of Antibody Binding Solution to the column. Any volume may be applied provided the total amount of IgG is ≤ 10mg.

Procedure for Solid-Phase Biotinylation

A. Antibody Binding

The antibody must be purified. If BSA is present in the antibody preparation, remove it before using this kit. See Appendix B for a list of suggested purification products.

1. Equilibrate Nickel-Chelated Column and PBS to room temperature before use.
2. To avoid introducing air bubbles into the column, open a Nickel-Chelated Column by first removing the top cap. Discard the storage solution (contains 0.01% sodium azide). Remove the column's bottom cap. Place column in a test tube.
3. Equilibrate column by adding 15mL of PBS and allowing the solution to drain through the column.
4. Apply the Antibody Binding Solution to the column and allow it to flow completely into the nickel-chelated resin. The column will stop flowing when the liquid level reaches the top disc.
5. Wash the column with 12mL PBS.

B. Antibody Biotinylation

1. Puncture the seal of one NHS-PEG₄-Biotin microtube with a pipette tip and dissolve tube contents in 200µL of PBS. Solubilize contents by gently pipetting up and down.
2. In a new test tube, make the appropriate NHS-PEG₄-Biotin solution for the amount of antibody being biotinylated as indicated in Table 1. Add PBS to the test tube first, and then add the biotinylation reagent.

Table 1. NHS-PEG₄-Biotin solution preparation.

<u>Antibody</u> <u>Amount (mg)</u>	<u>PBS</u> <u>Volume (mL)</u>	<u>Biotin</u> <u>Volume (µL)</u>	<u>Biotin Final</u> <u>Molarity (mM)</u>
1-2.5	3	88	0.5
2.6-4.5	3	141	0.8
4.6-5.9	3	200	1.1
6-7.5	2	200	1.7
7.6-10	1.5	200	2.5

3. Apply the biotin solution to the column.
4. After the solution has flowed completely through the nickel-chelated resin, cap the bottom and then the top of the column. Incubate for 30 minutes at room temperature.
5. After incubation, sequentially remove the top and bottom caps from the column. Wash the column with 15mL of PBS.

C. Antibody Elution

1. Place column in a new test tube. Add 3mL Elution Buffer to the column. Collect biotinylated antibody.
Note: Biotinylated antibodies are generally stable when stored in Elution Buffer (0.2M imidazole in PBS) at 4°C; however, stability is antibody-specific. Store biotinylated antibodies at -20°C if they will not be used within one month.
2. Regenerate the column by adding 3mL Elution Buffer.
3. For storage, wash the column with 15mL water containing 0.02% sodium azide. When approximately 3mL of solution remains above the top disc, replace the bottom cap followed by the top cap on the column and store upright at 4°C. When properly washed and stored, the column can be re-used up to 10 times without significant loss of binding capacity.

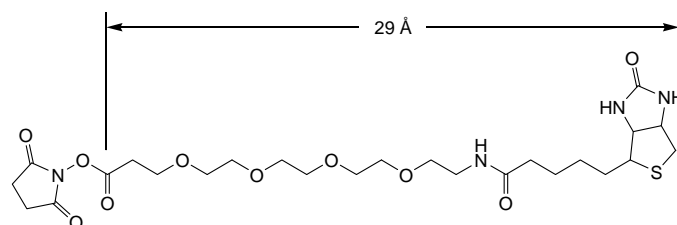
Troubleshooting

Problem	Cause	Solution
Antibody did not bind to column	Fab fragments, IgM or IgY were used	Do not use antibodies without an Fc region, or IgM or IgY with this kit
	BSA was present in antibody preparation	Remove BSA before using this kit
Antibody was not biotinylated	NHS-PEG ₄ -Biotin hydrolyzed before use	Reconstitute NHS-PEG ₄ -Biotin immediately before use and always use a new tube of biotinylation reagent for each reaction

Appendix

A. Structure of NHS-PEG₄-Biotin

The NHS ester of NHS-PEG₄-Biotin reacts with amines by nucleophilic attack forming an amide bond. The hydrophilic polyethylene glycol (PEG) spacer arm (29Å) imparts water solubility that is transferred to the biotinylated antibody, thus NHS-PEG₄-Biotin reduces aggregation of labeled antibodies stored in solution.



B. Bovine Serum Albumin (BSA) Removal

Two methods exist for removing BSA and/or gelatin from antibody preparations. The first is to affinity purify the antibody using immobilized Proteins A, G or L. Antibody will bind to the immobilized protein, allowing BSA to be removed by washing. The antibody is eluted and the solution is adjusted to a neutral pH (according to the protocol). Dilute the eluted antibody 1:1 with PBS before adding to the immobilized protein. For more information about Protein A, G, and L binding characteristics, see the catalog or Tech Tip #34 from our website.

The second method is to use Thermo Scientific Melon Gel Resin (e.g., Product No. 45206), which will bind to the BSA and gelatin and allow the purified antibody to be recovered in the flow-through. For more information about Melon™ Gel Products and this method of removal, see Tech Tip #55 from our website.

Related Thermo Scientific Products

28005	Biotin Quantitation Kit
23236	Coomassie Plus (Bradford) Protein Assay
21126	Streptavidin, Horseradish Peroxidase Conjugated, 1mg
21324	Streptavidin, Alkaline Phosphatase Conjugated, 1mg
15120	Pierce Streptavidin Coated Plates, 5 plates (see catalog for a complete listing of plates)

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