INSTRUCTIONS

Pierce Antibody Biotinylation Kit for IP

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90407

Number	Description
90407	**Pierce Antibody Biotinylation Kit for IP**, sufficient reagents for 8 labeling reactions, each containing 50-200µg of antibody in 100µL reaction volumes

Kit Contents:

EZ-Link NHS-PEG$_4$-Biotin, No-Weigh Format, 8 × 0.5mg microtubes
Molecular Weight: 588.67
Spacer Arm: 29Å

20X Phosphate Buffered Saline, 15mL

Zeba Spin Desalting Column (7K MWCO), 0.5mL, 8 columns, for 30-130µL samples

Storage: Upon receipt store at 4°C. Product is shipped with an ice pack.

Introduction

The Thermo Scientific™ Pierce™ Antibody Biotinylation Kit for IP provides optimized reagents for labeling antibodies and desalting columns for purifying the labeled molecule. Each reaction is sufficient for labeling 50-200µg of antibody in 100µL reaction volumes. The hydrophilic polyethylene oxide (PEO), also called polyethylene glycol (PEG), spacer arm imparts water solubility that is transferred to the biotinylated molecule. Consequently, antibodies labeled with NHS-PEG$_4$-Biotin exhibit less aggregation when stored in solution compared to antibodies labeled with reagents having only hydrocarbon spacers. This kit is specifically optimized to label antibodies for downstream immunoprecipitation (IP) applications involving streptavidin magnetic or agarose supports while having little to no effect on antigen binding. The NHS-PEG$_4$-Biotin is provided in Thermo Scientific™ No-Weigh™ Format as convenient single-use microtubes, eliminating difficulties associated with weighing small quantities of reagent.

Biotin is a small, naturally occurring vitamin that binds with high affinity to avidin and avidin-like proteins. Biotinylated antibodies typically retain biological activity because the biotin group is relatively small. An antibody conjugated with several biotin molecules can interact rapidly and tightly with streptavidin, making this a good platform for IP applications.

N-Hydroxysuccinimide (NHS) esters are the most popular biotinylation reagents. In pH 7-9 buffers, NHS esters react efficiently with primary amino groups (-NH_2) by nucleophilic attack, forming an amide bond and releasing the NHS (Figure 1). Proteins typically have many sites for labeling, including the primary amine in the side chain of lysine (K) residues and the N-terminus of each polypeptide.

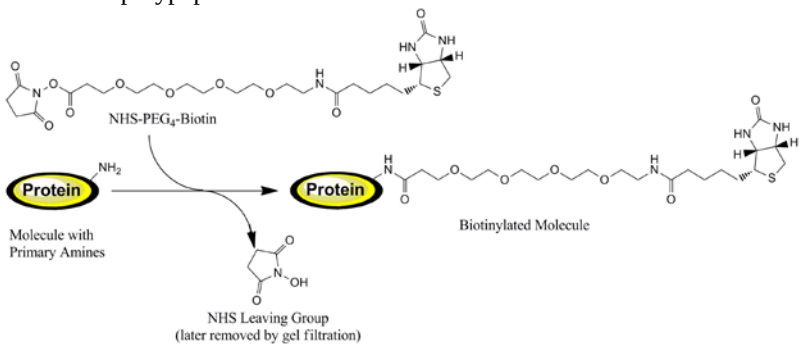

Figure 1. Reaction of NHS-PEG$_4$-Biotin with primary amine on an antibody. The leaving group and any non-reacted biotin reagent are removed during the desalting step.

Important Product Information

- Use reconstituted NHS-PEG_4-Biotin immediately. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, do not prepare solutions for storage. Discard any unused reconstituted reagent.
- NHS-PEG_4-Biotin is moisture-sensitive. Immediately before use, puncture the microtube foil with a pipette tip, add solvent and mix by pipetting up and down. After use, cut off the used microtube and discard. Store the microtube strip at 4°C in the foil pouch provided.
- Avoid buffers containing primary amines (e.g., Tris or glycine), as these will compete with the intended reaction (Figure 1). If necessary, dialyze or desalt to exchange the antibody into phosphate-buffered saline (PBS).
- Antibodies must be carrier-free (i.e., free of BSA and/or gelatin). Any carrier will compete with the intended reaction. To remove carrier, Protein A, G, or A/G resins may be used, as well as other commercially available antibody clean-up kits.
- This kit has been successfully used to label multiple mouse IgG_1, mouse IgG_2, rabbit monoclonal and polyclonal antibodies without impairing antigen binding. The number of biotin groups per antibody molecule ranges from 3-7, as determined by the Thermo Scientific™ Fluorescence Biotin Quantitation Kit (Product No. 46610).

Procedure for Biotinylating Antibodies

Note: The maximum total reaction volume must not exceed 110µL; this is the volume limit on the Thermo Scientific™ Zeba™ Spin Desalting Column.

A. Calculations

1. Calculate millimoles of NHS-PEG_4-Biotin to add to the reaction for a 40-fold molar excess:

$$\mu g\ IgG \times \frac{1 mg}{1000\ \mu g} \times \frac{1\ mmol\ IgG}{150{,}000\ mg\ IgG} \times \frac{40\ mmol\ Biotin}{1\ mmol\ IgG} = mmol\ Biotin$$

   - 40 = Recommended molar fold excess of biotin per protein sample
   - 150,000 = Molecular weight of IgG

2. Calculate microliters of 8.5mM NHS-PEG_4-Biotin (prepared in Step B.3) to add to the reaction:

$$mmol\ Biotin \times \frac{589\ mg}{1\ mmol\ Biotin} \times \frac{100\ \mu L}{0.5\ mg} = \mu L\ Biotin\ Solution$$

   - 589 = Molecular weight of NHS-PEG_4-Biotin
   - 100 = Microliters of solvent in which 0.5mg of NHS-PEG_4-Biotin is dissolved to make 8.5mM

Example: For 85µg of a 1mg/mL IgG (150,000 MW) solution, 2.7µL of 8.5mM NHS-PEG_4-Biotin will be added.

$$85\ \mu g\ IgG \times \frac{1 mg}{1000\ \mu g} \times \frac{1\ mmol\ IgG}{150{,}000\ mg\ IgG} \times \frac{40\ mmol\ Biotin}{1\ mmol\ IgG} = 0.000023 mmol\ Biotin$$

$$0.000023 mmol\ Biotin \times \frac{589\ mg}{1 mmol\ Biotin} \times \frac{100\ \mu L}{0.5\ mg} = 2.7\ \mu L\ Biotin\ Solution$$

3. Calculate the volume of 1X PBS to bring the total reaction to 100µL:
   100µL — (volume of antibody + volume of biotin) = volume of 1X PBS

B. Biotin-labeling Reaction

Note: The antibody must be in an amine-free buffer (preferably PBS) without carrier to perform this labeling reaction (see Important Product Information).

1. Dilute the 20X PBS to 1X (10mM sodium phosphate, 0.15M NaCl, pH 7.5) with ultrapure water. The 1X PBS will be used to equilibrate the Zeba Spin Desalting Column.

   Note: If precipitate formed in the 20X PBS, warm solution to 37°C in a water bath and vortex before use. The PBS can be stored at 1X if desired.
2. Cut off one microtube of NHS-PEG_4-Biotin from the No-Weigh Microtube Strip. Return the unused strip of microtubes to its pouch and store desiccated at 4ºC.
3. With a pipette tip, puncture the foil top on the biotin reagent microtube. To prepare 8.5mM solution, add 100µL of 1X PBS to the tube and mix by pipetting up and down several times.
4. Combine antibody, 1X PBS and NHS-PEG_4-Biotin (as calculated in Section A) and mix by gently pipetting up and down.
5. Incubate the reaction at room temperature for 30 minutes.

C. Buffer Exchange and Excess Biotin Removal

1. Remove bottom closure of Zeba Spin Desalting Column and loosen cap (do not remove cap).
2. Place column in a 1.5-2.0mL collection tube. Centrifuge at 1,500 × *g* for 1 minute to remove storage solution.
3. Place a mark on the side of the column where the compacted resin is slanted upward. Place column in the microcentrifuge with the mark facing outward in all subsequent centrifugation steps.
4. Add 300µL of 1X PBS on top of the resin bed. Centrifuge at 1,500 × *g* for 1 minute to remove buffer.
5. Repeat Step 4 two additional times, discarding buffer from the collection tube.
6. Place column in a new collection tube, remove cap and apply labeling reaction sample (from Section B) to the top of the compact resin bed.
7. Centrifuge at 1,500 × *g* for 2 minutes to collect the sample. Discard desalting column after use.
8. Store biotinylated antibody at 4°C for < 1 month. For longer periods, store at -20°C or -80°C.

Troubleshooting

Problem	Possible Cause	Solution
Low or no biotinylation	Buffer contained primary amines	Buffer exchange the antibody into a non-amine-containing buffer such as the PBS provided by desalting columns or dialysis
	NHS-PEG_4-Biotin was hydrolyzed	Use reagent immediately upon reconstitution
	Carrier protein was present in the antibody solution	Remove carrier protein before biotinylation by using Protein A, G or A/G resin or an antibody clean-up kit. This will reduce competition for labeling

Additional Information

Please visit our website for additional information including the following:

- Tech Tip #43: Protein stability and storage

Related Thermo Scientific Products

90408	**Pierce™ MS-Compatible Magnetic IP Kit (Streptavidin)**
88816	**Pierce™ Streptavidin Magnetic Beads,** 1mL
89882	**Zeba Spin Desalting Columns, 7K MWCO, 0.5mL**
69570	**Slide-A-Lyzer™ MINI Dialysis Device, 10K MWCO, 0.1mL**
46610	**Fluorescence Biotin Quantitation Kit**
20423	**Pierce™ Protein A/G Plus Agarose Resin**

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