EZ-Link™ NHS-PEG Solid-Phase Biotinylation Kit – Mini-Spin Columns

Catalog Numbers 21450

Doc. Part No. 2161474 Pub. No. MAN0011501 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Thermo Scientific™ EZ-Link™ NHS-PEG Solid-Phase Biotinylation Kit allows for efficient biotinylation of purified IgGclass antibodies. This solid-phase biotinylation method uses Ni-NTA agarose resin to first immobilize purified IgG. The antibody is then biotinylated by adding a solution of NHS-PEG_4-Biotin. Excess biotin is washed from the column, and the antibody is eluted in a buffered imidazole solution. The reaction results in ~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 Cγ2 and Cγ3 domains that is highly conserved across all mammalian IgGs (1–4). Purified IgG from sheep, mouse, goat, rat and rabbit will bind to nickel-chelated resin.

This kit includes Thermo Scientific™ No-Weigh™ NHS-PEG_4-Biotin packaged in convenient pre-measured microtubes, eliminating difficulties associated with weighing small quantities of reagent. NHS-PEG_4-Biotin (see Figure 1 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-PEG_4-Biotin imparts water solubility that is transferred to the biotinylated antibody, thus NHS-PEG_4-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 protocol completion is comparable to solution-phase protocols, antibody immobilization eliminates the need for desalting or dialysis to remove excess biotin, resulting in excellent antibody recovery.

Contents and storage

Product	Catalog No.	Components	Amount	Storage
EZ-Link™ NHS-PEG Solid-Phase Biotinylation Kit – Mini-Spin Columns[1]	21450	HisPur™ Ni-NTA Spin Columns, 0.2 mL resin bed	8 columns	4°C
		No-Weigh™ NHS-PEG_4-Biotin, 2 mg microtubes	8 tubes	
		BupH™ Phosphate Buffered Saline Pack	1 pack	
		4 M Imidazole Stock Solution	5 mL	
		Pierce™ Microcentrifuge Tubes, 2 mL	30 tubes	

[1] Sufficient materials for 8 biotinylation reactions each consisting of 100–1,000 µg IgG.

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 an Fc region, as they do not bind efficiently to the nickel-chelated agarose.
- This protocol has been optimized for 0.1–1 mg of antibody. The antibody preparation must be free of chelating agents such as EDTA and EGTA.

- Bovine serum albumin (BSA) 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 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-PEG_4-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 (Cat. No. 28005); however, 0.2 M imidazole (Elution Buffer) interferes with the HABA assay. Dilute on-column biotinylated IgG 1:1 with phosphate-buffered saline (PBS) before use in the HABA assay to reduce imidazole concentration to 0.1 M.
- Protein assays can be used to determine concentration of eluted IgG. When determining concentration of IgG in Elution Buffer, use Coomassie Plus (Bradford) Assay Reagent (Cat. No. 23236). The Pierce™ BCA Protein Assay Kit cannot be used because imidazole interferes with the assay chemistry.

Required materials not provided

- 0.2 µm, 500 mL filter sterilization unit
- Test tubes and test tube rack
- Rotating platform or microcentrifuge tube nutator

Before you begin

Prepare PBS

1. Reconstitute contents of the Thermo Scientific BupH Phosphate Buffered Saline (PBS) pack with 500 mL of ultrapure water.
2. Filter-sterilize solution using a 0.2 µm-filter apparatus and store at 4°C.

When stored properly, there is sufficient buffer for 8 antibody biotinylation reactions of up to 1 mg of IgG each.

Prepare Elution Buffer

Prepare 1 mL of Elution Buffer by diluting 50 µL of the 4 M Imidazole Stock Solution with 950 µL of PBS.

Prepare Antibody Binding Solution

Adjust the volume of purified IgG (0.1–1 mg) with PBS to 500 µL to 1 mL.

Note: The volume of the Antibody Binding Solution will depend on the antibody concentration. Use the lowest possible volume (500 µL) to maximize antibody binding. Volumes greater than 1 mL can be used, but decreased binding efficiency will result.

Perform solid-phase biotinylation

Equilibrate the HisPur™ Ni-NTA Resin

1. Remove the bottom tab from the HisPur™ Ni-NTA Spin Column by gently twisting. Place column into a centrifuge tube.

   Note: Use 2-mL centrifuge tubes for the 0.2-µL spin column.
2. Centrifuge column at 700 × *g* for 2 minutes to remove storage buffer. Discard the flowthrough.
3. Equilibrate column with two resin-bed volumes of PBS. Allow buffer to enter the resin bed.
4. Centrifuge column at 700 × *g* for 2 minutes to remove storage buffer. Discard the flowthrough.
5. Place the bottom plug in the column and proceed immediately to step 1 in “Bind the antibody” on page 3.

Bind the antibody

The antibody must be purified. If BSA is present in the antibody preparation, remove it before using this kit. See “Bovine Serum Albumin (BSA) removal” on page 5 for a list of suggested purification products.

1. Add the prepared Antibody Binding Solution to the HisPur™ Ni-NTA Spin Column. Insure the bottom plug and cap are securely fastened.
2. Invert tube several times to suspend the resin. Incubate 10 minutes at room temperature with gentle rocking motion on a rotating platform. **DO NOT VORTEX.**

   Note: The resin must remain suspended during binding. If necessary, invert the tube manually every 2–3 minutes to keep the resin in suspension.
3. Remove the bottom plug. Centrifuge the column in a centrifuge tube at 700 × *g* for 2 minutes and discard the flowthrough.
4. Add 0.5 mL of PBS to the tube. Invert tube several times to wash the resin.
5. Centrifuge the column in a centrifuge tube at 700 × *g* for 2 minutes and discard the flowthrough.
6. Repeat steps 4 and 5 three additional times to complete washing and proceed immediately to step 1 in “Biotinylate antibody” on page 3.

Biotinylate antibody

1. Apply bottom plug to column.
2. Immediately before use, unscrew the No-Weigh™ microtube and dissolve the tube contents by adding 200 µL of PBS. Gently pipette up and down to mix.
3. Add 190 µL of PBS to the column.
4. Add 10 µL of NHS-PEG_4-Biotin to the column.
5. Cap top of column with a screw cap. Mix by gentle flicking.
6. Incubate for 30 minutes at room temperature.

   Note: Flick the column occasionally during incubation to keep the resin from settling. **DO NOT VORTEX.**
7. Remove the bottom plug. Centrifuge the column at 700 × *g* for 2 minutes and discard the flowthrough.
8. Add 400 µL of PBS to the column. Centrifuge the column at 700 × *g* for 2 minutes and discard the flowthrough.
9. Repeat step 7 three additional times to wash the column.

Elute antibody

1. Cap bottom of column. Place column in a new 2-mL microcentrifuge tube.
2. Add 200 µL of Elution Buffer to the column and incubate for 10 minutes at room temperature.
3. Elute antibody from the resin by centrifugation at 700 × *g* for 2 minutes.

   Note: After elution, some antibody will remain bound to the column. To increase the yield of biotinylated antibody, repeat steps 2 and 3, collecting each fraction in a separate tube. To increase concentration of smaller amounts of antibody (i.e., 0.1–0.25 mg), re-apply eluted antibody solution to the column and repeat step 3. Discard the resin after use.
4. Store biotinylated antibody at 4°C for up to one month.

   Note: Biotinylated antibodies are generally stable when stored in Elution Buffer (0.2 M Imidazole in PBS) at 4°C; however, stability will depend on the specific antibody being used. If biotinylated antibodies are not to be used within one month, store them in single-use volumes at –20°C.

Troubleshooting

Observation	Possible cause	Recommended action
Antibody does not bind to column	BSA was present in antibody preparation.	Remove BSA before using the kit.
	Fab fragments, IgM or IgY were used.	Do not use antibodies without an Fc region, IgM, or IgY with the kit.
Antibody is 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.

Related products

Product	Catalog No.
Pierce™ Biotin Quantitation Kit	28005
Pierce™ Coomassie Plus (Bradford) Assay Kit	23236
Pierce™ Microcentrifuge Tubes	69715
Streptavidin, Horseradish Peroxidase Conjugated	21126
Streptavidin, Alkaline Phosphatase Conjugated	21324
Streptavidin Coated Plates	15120

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General references

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Appendix

NHS-PEG$_4$-Biotin structure

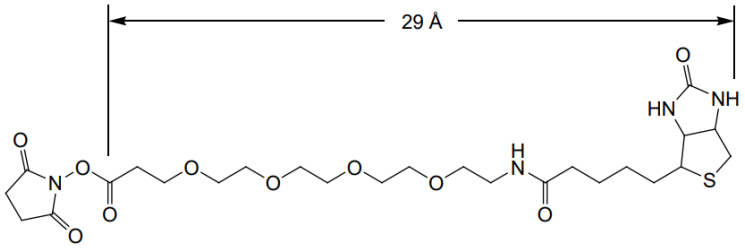

Figure 1 The NHS ester of NHS-PEG$_4$-Biotin reacts with amines by nucleophilic attack forming an amide bond. The hydrophilic polyethylene oxide (PEG) spacer arm (29 Å) imparts water solubility that is transferred to the biotinylated antibody, thus NHS-PEG$_4$-Biotin reduces aggregation of labeled antibodies stored in solution.

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 HisPur™ Ni-NTA Spin Column. For more information about Protein A, G, and L binding characteristics, visit our website.

The second method is to use Thermo Scientific™ Melon™ Gel Resin (e.g., Cat. No. 45206), which will bind to the BSA and gelatin and allow the purified antibody to be recovered in the flowthrough. For more information about Melon™ Gel products and this method of removal, visit our website.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision history: Pub. No. MAN0011501

Revision	Date	Description
B.0	30 June 2022	Updating user guide to current formatting standards.
A.0	17 October 2015	Initial release.