

NHS-Biotinylation Kit

S1510182

Storage: Room Temperature. 2-8°C. -20°C. Protect from light. For detailed storage information, please refer to the kit contents.

Shipping: Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

The NHS-Biotinylation Kit is designed for efficiency and reliability, delivering ready-to-use labeled antibody in just 60 minutes, with minimal hands-on time. The kit include all necessary reagents for antibody labeling, including Biotin-NHS, buffers, purification resin, and empty spin columns. The Biotin-NHS provided in the kit is suitable for labeling antibodies, proteins, and other molecules that have primary amines. NHS-activated biotins react efficiently with primary amino groups ($-NH_2$) in pH 7-9 buffers to form stable amide bonds (Fig 1). Spin columns included in the kit are used for purifying the biotinylated antibody from excess biotin reagent with yields of 70-95%. The kit contains sufficient reagents for 10 biotinylation reactions of 1 mg of antibody. The biotinylated antibody can be detected in ELISA, Western Blot, IF/ICC or Flow Cytometry application using avidin or streptavidin probes. Aladdin offers a series of high-performance streptavidin conjugates designed for detecting the performance of biotinylated antibodies.

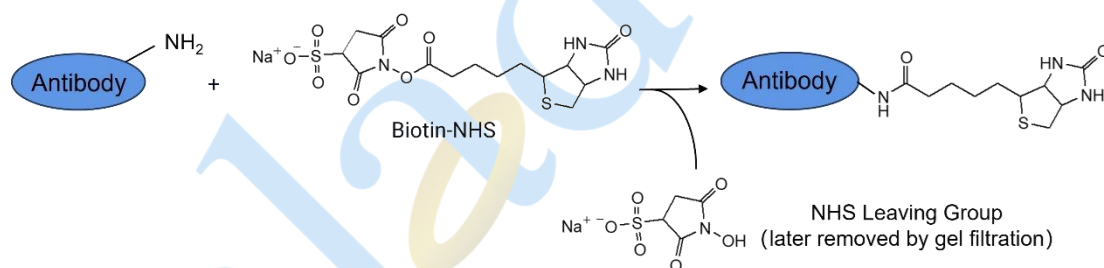


Fig. 1 NHS-Biotinylation Kit (S1510182) Labeling Principle Schematic

Required materials not supplied

1. Microcentrifuge capable of $1000 \times g$.
2. Desired antibody for labeling (free of BSA or any carrier protein).
3. PBS buffer (pH 7.2-7.4).

Kit Contents

S1510182	Component	10 reactions	Storage	Quantity Per reaction
S1510182A	Biotin-NHS	10 vials	-20°C. Store in the dark.	1 vial for labeling 1 mg of antibody
S1510182B	NaHCO ₃	500 mg	RT.	Prepare according to instructions
S1510182C	DMSO	0.5 mL	RT.	Prepare according to instructions
S1510182D	Purification Resin	30 mL	2-8°C. Do not freeze.	3 mL for 1 reaction
S1510182E	Empty Spin Column	10 EA	RT.	1 EA for 1 reaction

Important

1. The purified antibody should be in a buffer that does not contain primary amines (for example, ammonium ions, Tris, glycine, ethanolamine, triethylamine, glutathione) or imidazole. All of these substances significantly inhibit protein labeling.
2. Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.
3. An antibody concentration of 1-2 mg/mL is optimal. Concentrations below 1 mg/mL may reduce labeling efficiency, while excessively high concentrations may result in too small a volume of antibody, which is unfavorable for purification recovery.
4. The volume of the antibody to be labeled should be controlled between 0.4 mL and 0.7 mL to ensure optimal removal efficiency using the purification column.
5. Before formal experimentation, the reagents must be returned to room temperature and centrifuged before use. Both lower temperatures and reagent residue on the centrifuge tube walls may impair conjugation efficiency.
6. The purification resin included in this kit is recommended for single use only.
7. During operation, always wear a lab coat, disposable gloves, and protective equipment.
8. All products are for research use only.

Instruction for use

1. Prepare the reagents
 - 1.1 Prepare a 1M NaHCO₃ solution-Prepare an appropriate amount of 1M NaHCO₃ solution based on the sample volume. For example, weigh 42 mg of NaHCO₃ and add 0.5 mL of ultrapure water to obtain a 1M NaHCO₃ solution. NaHCO₃ can maintain the pH of the labeling reaction system between 7-9, thereby improving labeling efficiency.
Note: The NaHCO₃ solution must be prepared fresh before each use.
2. Label the antibody
 - 2.1 If the antibody to be labeled has a concentration of ≥1 mg/mL and is in an appropriate

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buffer, add 10% of the antibody volume of 1 M NaHCO_3 . If the antibody is a powder lyophilized from an appropriate buffer, dissolve 1 mg of the antibody in 0.4-0.7 mL of 0.1 M NaHCO_3 . Prepare 0.1 M NaHCO_3 buffer by diluting the 1 M solution 10-fold with ultrapure water.

- 2.2 Biotin-NHS Preparation: Add 20 μL of DMSO to the Biotin-NHS reagent tube and vortex to ensure the biotin is completely dissolved.

Note: To visually ensure that the dye has fully dissolved, peel the label off the vial of biotin reagent.

- 2.3 Add prepared antibody solution to the vial of biotin reagent. Cap the vial and gently invert it a few times to fully dissolve the biotin reagent.

- 2.4 Incubate the solution for 60 minutes at room temperature. The reaction can be carried out on a shaker or mixer, recommended speed for flipping up and down is 25 rpm. If a mixing instrument is not used during the reaction process, the reaction solution should be mixed upside down every 10 minutes.

Note: During the incubation period, proceed to steps 3 below, to prepare a spin column for the purification of the labeled protein.

3. Prepare the spin column

- 3.1 Place an empty spin column in a 15 mL collection tube.

- 3.2 Suspend the purification resin by repeated inversion of the reagent bottle. Then add 3.0 mL of the suspension into the column and allow the resin to settle. Allow the column buffer to drain from the column by gravity. Initially, some pressure may be required to cause the first few drops of buffer to elute. Centrifuge the column at $1000 \times g$ for 2 minutes, discard the storage buffer and return column to the same collection tube.

Note: The purification resin is supplied as a slurry in 20% ethanol, with a resin-to-ethanol volume ratio of 3:1 (v/v).

- 3.3 Add 2 mL of PBS Buffer, then centrifuge the column-tube assembly at $1000 \times g$ for 2 minutes to equilibrate the column.

Note: When using a fixed-angle rotor, place a mark on the side of the column that faces away from the rotor center. For all subsequent centrifugation steps, place the column in the microcentrifuge with the mark facing away from the rotor center.

- 3.4 Repeat step 3.3 for two additional times. Add PBS for the third wash but wait to centrifuge until immediately prior to proceeding to step.

- 3.5 After the third wash check there is no solution in the column, if required briefly centrifuge until no solution remains.

4. Purification with Desalting Column

- 4.1 Insert prepared desalting column into the new Collection Tube and apply the entire sample from step 2.3 to the column.

- 4.2 Centrifuge the column-tube assembly at $1000 \times g$ for 2 minutes. The purified antibody conjugate is in the collection tube.

5. Determine the antibody concentration (Optional)

5.1 Determination of antibody concentration by measuring absorption at 280 nm.

6. Storage

6.1 Add 0.05-0.2% Proclin 300 or 0.05% sodium azide, along with a protein stabilizer (such as 0.1% BSA), to the labeled protein. Store protected from light at 2-8°C for stable preservation up to six months. Alternatively, add an equal volume of glycerol and store at -20°C for stable preservation up to six months.

