Micro NHS-PEG4-Biotinylation Kit

M1510180

Storage: Room Temperature. 2-8℃. -20℃. 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-PEG4-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-PEG4-NHS, buffer, and purification columns. The Biotin-PEG4-NHS reagent included in the kit is a pegylated, water-soluble reagent 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). Meanwhile, pegylation imparts water solubility to the biotinylated molecule, helping to prevent aggregation of biotinylated antibodies stored in solution. 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 100 µg 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.

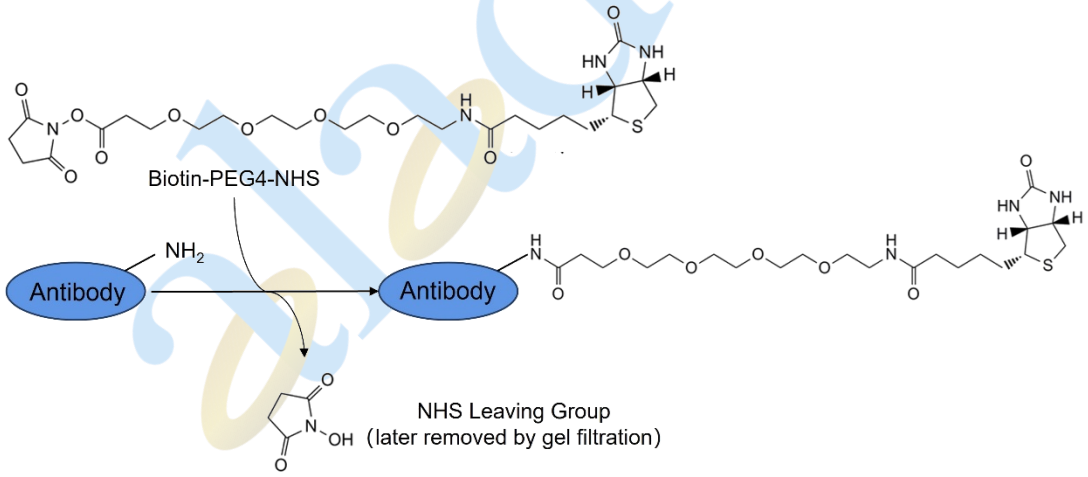

Fig. 1 Micro NHS-PEG4-Biotinylation Kit (M1510180) Labeling Principle Schematic

Kit Contents

M1510180	Component	10 reactions	Storage	Quantity Per reaction
M1510180A	Biotin-PEG4-NHS	10 vials	-20℃. Store in the dark.	1 vial for labeling 100 μg of antibody
M1510180B	$NaHCO_3$	100 mg	RT.	Prepare according to instructions
M1510180C	Spin Desalting Column	10 EA	2-8℃. Do not freeze.	1 EA for 1 reaction
M1510180D	Collection Tube	10 EA	RT.	1 EA for 1 reaction

Required materials not supplied

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

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. Concentrate the antibody to ≥1 mg/mL.
4. Do not reuse the purification resin.
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 reactive dyes should be protected from prolonged exposure to light.
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 $NaHCO_3$ solution-Prepare an appropriate amount of 1M $NaHCO_3$ solution based on the sample volume. For example, weigh 42 mg of $NaHCO_3$ and add 0.5 mL of ultrapure water to obtain a 1M $NaHCO_3$ solution. $NaHCO_3$ can maintain the pH of the labeling reaction system between 7-9, thereby improving labeling efficiency. Note: The $NaHCO_3$ 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

buffer, dilute it to 1 mg/mL, and add a 10% volume of 1M $NaHCO_3$. If the antibody is a powder lyophilized from an appropriate buffer, prepare a 1 mg/mL solution of the antibody by adding an appropriate amount of 0.1M $NaHCO_3$ to the antibody. Prepare 0.1M $NaHCO_3$ by diluting the 1M solution 10-fold with ultrapure water.

2.2 Transfer 100 µL of the antibody solution to the vial of biotin reagent. Cap the vial and gently invert it a few times to fully dissolve the biotin reagent.

2.3 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 Loosen the cap on a spin column, twist the tab off of the bottom, then place the column into a collection tube. Centrifuge the column-tube assembly at 1000 × g for 2 minutes to remove the storage solution. Discard the flowthrough, then place the column back into the collection tube.

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.

3.2 Add 500 µL of PBS Buffer, then centrifuge the column-tube assembly at 1000 × g for 2 minutes to equilibrate the column.

3.3 Repeat step 3.2 for two additional times. Add PBS for the third wash but wait to centrifuge until immediately prior to proceeding to step.

3.4 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 × 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. Strorage

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.