Protein Biotinylation Kit

Catalog Number: CO2028

For Research Use Only. Not for use in Diagnostic Procedures.

1. Intended Use

Kit provides rapid, simple, and reliable components for the labeling of proteins for use in lateral flow procedures. Kit enables the biotinylation of antibody or proteins to be used with the Universal Lateral Flow Kits (AU2034, AU2037 or AU2044 in addition to Streptavidin Colloidal (AU2017) gold or Streptavidin CdZnS Quantum Dots (AU2043).

2. Introduction

Attogene's Protein-Biotinylation kit provides the user with reagents for labeling proteins, desalting columns for purifying the labeled molecule, and convenient and easy to interpret instructions on how to carry out the labeling process. You can expect each reaction to carry out sufficient labeling of 50ug to 200ug of sample in 100ul of sample volumes. The polyethylene glycol spacer arm, commonly referred to as PEG, bestows water solubility that is transferred to the biotinylated molecule. Compared to antibodies labeled with reagents only having hydrocarbon spacers, there is significantly less aggregation displayed when stored in solution. We have optimized this kit to specifically work in lateral flow applications involving streptavidin while having little to no effect on antigen binding. We provide the NHS-PEG_4-Biotin conveniently weighed out in .5mL tubes alleviating the difficulties associated with weighing out and consequently providing stable storage of the reagent.

3. Kit Contents

Component Name	Volume	Storage
0.5mL Micro Spin Desalting Columns	8x	2 - 4°C
0.5mg NHS-Peg_4-Biotin	8 0.5mL tubes	2-4°C stored with desiccant
15mL of 20X PBS	1 Container	2-4°C
1 instruction booklet	1	RT

4. Storage and Stability

- The kit should be stored at 2°C - 4°C until ready to use.

5. Required Materials Not Supplied

- 1.5mL or 2.0 mL microcentrifuge tubes
- Variable-Speed Microcentrifuge

6. Precautions

- You must use the reconstituted biotin immediately. Degradation of the NHS-ester moiety occurs due to it hydrolyzing and therefore becoming non-reactive. Do not prepare any solutions for storage. Once your reconstituted reagent has been used, discard the solution.
- NHS-PEG_4-Biotin is extremely moisture sensitive. Therefore, it is imperative that once you remove the cap you must immediately add your 1X PBS. Pipette your solution up and down until fully dissolved. Discard tube after use.
- Avoid any buffers containing primary amines. If present, these will compete with the primary reaction. We recommend desalting to exchange the antibody into PBS using Attogene Desalting Columns (CO2027).
- Your protein must be carrier-free (i.e., free of BSA and/or gelatin). Like primary amines, any carriers present will also compete with the intended reaction. Protein cleanup kits may be used to purify antibodies.

8. Procedure

Caution: The maximum sample volume of Attogene's Micro Spin Columns (CO2027) is 130ul.

A. Calculations

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

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

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

2. Calculate microliters of 8.5mM NHS-PEG_4-Biotin to add to the reaction.

$$\text{mmol Biotin} \times \frac{589\text{mg}}{1\text{mmol Biotin}} \times \frac{100\text{ul}}{1\text{mmol IgG}} = \text{ul Biotin Solution}$$

- 589 = The molecular weight of NHS-PEG_4-Biotin
- 100= ul of solvent/biotin solution 8.5mM

B. Biotin-Labeling Reaction

Note: The protein must be in an amine free and carrier free buffer, preferably PBS, prior to labeling.

1. Dilute your 20X PBS down to 1X (10mM sodium phosphate, .15M NaCl, PH 7.5) with ultrapure water. The 1x PBS will be used in the equilibration of the micro spin columns.
2. Remove one tube of NHS-PEG_4-Biotin from the pre-weighed tubes. Return unused tubes back to their container and store desiccated at 4°C.
3. Create your 8.5mM solution, add 100ul of 1X PBS to your tube and pipette up and down several times.
4. Combine your antibody and NHS-PEG_4-Biotin (as calculated in section A) and mix gently by pipetting up and down.
5. Incubate at room temperature for 30 minutes.

C. Buffer Exchange Instructions:

Equipment Required:

- 1.5mL or 2.0 mL microcentrifuge tubes
- Variable-Speed Microcentrifuge

A. Spin Column Preparation

Step 1. Break away tab off from the bottom of the column and loosen cap on the top.

Step 2. Place column into a 1.5mL or 2mL centrifuge tube and run for one minute at 1,500rcf.

Step 3. Remove contents of the centrifuge tube and add 300ul of either PBS lacking preservation agent, HEPES, or another buffer of your choice. Make sure you blot the bottom of the tube and avoid touching the sides.

Step 4. Spin columns at 1,500rcf for one minute and repeat this step and step three two more times.

Step 5. 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. Improper orientation will result in reduced desalting efficiency.

B. Sample Loading

Step 1. Place your desalting column into a new centrifugation tube. Then apply 30-130uL of sample directly to the top of the resin bed.

Step 2. For sample volumes under 70uL apply a 15uL stacker of ultrapure water or buffer to the top of the gel bed after your sample has been fully absorbed. By doing so, this will ensure maximum protein recovery.

Step 3. Centrifuge at 1,500rcf for two minutes to collect your sample. Discard the used desalting column after use.

Step 4. Store biotinylated protein at 4°C for <1month. For longer periods store at -20°C or -80°C. A preservation agent, such as sodium azide, may be added at this stage to prevent any contaminating growth.

9. Trouble Shooting

Problem: Low Biotinylation

Possible Causes	Solutions
Buffer Contained Primary Amines	Make sure you buffer exchange the antibody into a non-amine-containing buffer like the PBS provided in the kit.
NHS-PEG_4-Biotin was hydrolyzed	Use the reagent immediately upon reconstitution.
Carrier protein was present in the antibody solution.	Purify the antibody by using protein A, G, or A/G resin or an antibody purification kit.