NHS-Biotin Labeling Kit

Product Code: OM700012

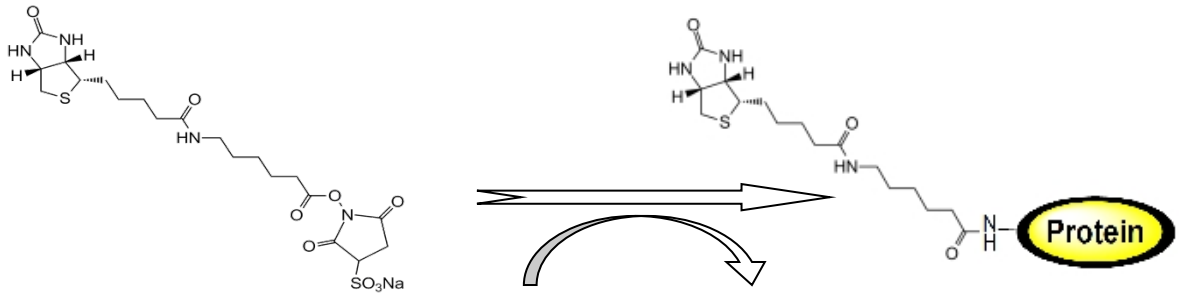
Package Contents:

Composition:	OM700012
A: Biotin（NHS-Biotin）	**1 vial**
B: Labeling Buffer Solution (PH=7.4)	**1 bottle**
C: Ultrafiltration Tube（10KDa）	**1 set**
D: Protein Storage Solution	**1 bottle**

Storage Conditions: Biotin powder: Store at -20°C. Other labeling components: Store at 4°C.

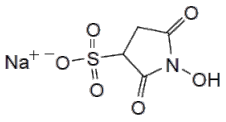
The labeling kit is transported with ice packs.

This biotin labeling kit provides all the reagents required for rapid biotin labeling, designed for the labeling of antibodies, proteins, peptides, or other macromolecules containing primary amino groups (-NH2). The activated biotin supplied in this kit can label approximately 10 mg of IgG antibody. Users can calculate the required amount of biotin to be added based on their specific needs.



NHS-Biotin　　Protein-Biotin





Amino-protein　　Reaction by-Product

Experimental Preparation:

1. Carefully read the instruction manual.
2. Remove all components of the labeling kit from the refrigerator in advance and allow them to equilibrate to room temperature.
3. Dissolve the biotin in Component A by adding 180 μL of Component B, and mix thoroughly to prepare a 10 mM biotin working solution.
4. Calculate the required amount of biotin to be added based on the labeling requirements.
5. **Calculation of Biotin Labeling Amount:**

- The degree of labeling depends on the distribution of amino groups on the protein, protein concentration, and the amount of reagent used.
- Experimental analysis indicates that when the concentration of the target protein or antibody is 2 mg/mL, a 20-fold molar excess of biotin (relative to the protein/antibody) should be used.
- If the concentration is below 1 mg/mL, a higher molar ratio of biotin must be added to ensure sufficient labeling of the protein or antibody molecules.

Use the following formula to calculate the required molar amount of biotin to be added:

mL protein × (mg protein/mL protein) × (mmol protein/mg protein) × (mmol Biotin/mmol protein) × 20 = mmol Biotin

Then calculate how many microliters (μL) of 10 mM biotin are needed:

mmol Biotin × (1000000μL/L) × (L/10mmol) = μL Biotin

Example: There is 1mL of 2mg/mL IgG solution to be labelled, then ~27μL of 10mM NHS-Biotin needs to be added.

1mL IgG × 2mg/mL IgG × (1mmol IgG/150000 mg IgG) × (1mmol Biotin/1mmol IgG) × 20 = 0.000267 mmol Biotin

0.000267 mmol Biotin × (1000000μL/L) × (L/10mmol) = 26.7μL Biotin

Protocol Steps (for labeling 1 mg of antibody):

1. **Preparation of Antibody Solution**
   - Take 1 mg of the target antibody and place it in the Component C ultrafiltration tube.
   - Add an appropriate volume of Component B to adjust the antibody concentration to ~2 mg/mL.
     (If the antibody is too dilute, concentrate it via ultrafiltration centrifugation. If the sample contains interfering substances like free Tris, amino acids, or other primary amine-containing compounds, wash repeatedly with Component B to ensure complete removal.)
2. **Biotin Labeling Reaction**
   - Add 13.3 μL of 10 mM biotin working solution (prepared earlier) to the ultrafiltration tube.
   - Adjust the final volume to 0.5 mL with Component B and mix gently by pipetting.
   - Incubate at 37°C for 30 min, gently mixing every 10 min to ensure proper labeling.
     (Note: The 10 mM biotin working solution should ideally be used immediately after preparation.)
3. **Post-Labeling Verification (Optional)**
   - At this stage, the labeling is complete, and the biotinylated antibody can be tested directly by ELISA or Western blot (even though unreacted/hydrolyzed biotin may still be present in the solution).
4. **Removal of Excess Biotin**

- Add Component B to the ultrafiltration tube and centrifuge at 6,000 rpm for 10 min.
- Repeat this washing step at least 4 times to ensure complete removal of free biotin.

5. **Storage of Biotinylated Antibody**
   - Collect the final biotin-labeled antibody from the ultrafiltration tube.
   - For -20°C storage, mix with an equal volume of Component D (preservative/stabilizer).
   - For -80°C storage, mix with two-thirds volume of Component D for long-term preservation.

Important Notes:

1. **Applicability**
   - This kit is suitable for labeling any macromolecule containing primary amines (-NH_2), including proteins, antibodies, peptides, and other primary amine-containing molecules.
   - The optimal amount of biotin should be selected based on the molecular weight and desired degree of labeling.
2. **Storage Solution (Component D)**
   - The kit includes Component D (storage solution), which primarily contains glycerol.
   - Users may choose alternative storage buffers based on the specific characteristics and intended applications of their labeled molecules.
3. **Shelf Life**
   - The unopened kit is stable for 12 months—please use it before the expiration date.
4. **Ultrafiltration Tube (Component C) Specifications**
   - The provided ultrafiltration tube (Component C) has:
   - 10 kDa molecular weight cutoff (MWCO)
   - Maximum volume capacity of 0.5 mL
   - If your target molecule has a different molecular weight or requires larger labeling volumes, please select an appropriate ultrafiltration tube or contact us for customized specifications.
5. **Stability of Biotin Working Solution**
   - ***The 10 mM biotin working solution should ideally be used immediately after preparation.***
   - ***If storage is necessary, the remaining solution can be kept at -20°C for up to 1 week, but coupling efficiency may decrease over time.***