Protein Biotinylation Kit

Cat#: orb867216 (Protocol)

Component Storage Amount
Component A: Biotin SE Freeze (< -15 °C), Minimize light exposure 2 vials (lyophilized)
Component B: Reaction Buffer Refrigerated (2-8 °C), Minimize light exposure 1 vial (200 µL)
Component C: Spin Column Refrigerated (2-8 °C) 2 columns

OVERVIEW

Biotin is widely used for labeling biomolecules, in particular, antibodies. Our Protein Biotinylation Kit is optimized for the preparation of biotin-labeled IgG for enzyme immunoassay (EIA). It uses biotin succinimidyl ester that reacts with the amino groups of IgG and other biomolecules. This kit comes with all the necessary reagents for labeling and purification. On our hands, 3 to 6 biotin molecules can be conjugated to each IgG molecule depending on the molecular weight and lysine residues available on the IgG molecule. The kit can be used for 2 conjugation reactions. For each conjugation reaction the material can label up to 1 mg protein. The entire process only takes less than an hour.

AT A GLANCE

Protocol Summary

1. Add 20 µl Reaction Buffer (Component B) into target protein (200 µl)
2. Add the protein solution into Biotin SE (Component A)
3. Incubate at room temperature for 30-60 minutes
4. Purify the conjugate by spin column

Important Upon receipt, store Biotin SE (Component A) at -20oC, kept from moisture. Store other components at room temperature. Do not freeze Reaction Buffer (Component B) and Spin Column Component C). Warm all the components before opening, and immediately prepare the required solutions before starting the conjugation. You might need further optimization for your protein labeling since this SOP was developed for Goat anti-Rabbit IgG labeling.

PREPARATION OF WORKING SOLUTION

Protein working solution

For labeling 1 mg target protein (assuming the target protein concentration is 5 mg/mL), mix 20 µL (10% of the total reaction volume) of Reaction Buffer (Component B) with 200 µL of the target protein solution.

Note If you have a different protein concentration, adjust the protein volume accordingly to make ~1 mg protein available for this labeling reaction.

Note The pH of the protein solution should be 8.5 ± 0.5. If the pH of the protein solution is lower than 8.0, adjust the pH to the range of 8.0-9.0 using Reaction Buffer (Component B) or saturated sodium bicarbonate solution.

Note The protein should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2-7.4. If the protein is dissolved in Tris or glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation.

Note Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin might not be labeled well. The presence of sodium azide or thimerosal might also interfere with the conjugation reaction. Sodium azide or thimerosal can be removed by dialysis or spin column for optimal labeling results.

Note The conjugation efficiency is significantly reduced if the protein concentration is less than 2 mg/mL. For optimal labeling efficiency, the final protein concentration range of 2-10 mg/mL is recommended.

SAMPLE EXPERIMENTAL PROTOCOL

Run conjugation reaction

1. Add the protein working solution into the vial of Biotin SE (Component A), and mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Keep the conjugation reaction mixture at room temperature for 30 - 60 minutes.

Note The conjugation reaction mixture can be rotated or shaken for longer time if desired.

Prepare spin column for sample purification

1. Invert the provided spin column (Component C) several times to re-suspend the settled gel and remove any bubbles.
2. Snap off the tip and place the column in a washing tube (2 mL, not provided). Remove the cap to allow the excess packing buffer to drain by gravity to the top of the gel bed. If column does not begin to flow, push cap back into column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Washing Tube. However, centrifuge immediately if the column is placed into a 12 x 75 mm test tube (not provided).
3. Centrifuge for 1 minutes in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.
4. Apply 1-2 mL 1X PBS (pH 7.2-7.4) to the column. After each application of PBS, let the buffer drain out by gravity, or centrifuge the column for 2 minutes to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3-4 times.
5. Centrifuge for 2 minutes in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.

Purify the conjugates

1. Place the column (from Step Prepare spin column for sample purification) in a clean Collecting Tube (1.5 mL, not provided). Carefully load the sample (100-500µL, from Step conjugation reaction) directly to the center of the column.
2. After loading the sample, add 300 µL of 1X PBS (pH 7.2-7.4) to make the total volume of 220 µL to 520 µL. Centrifuge the column for 5-6 minutes at 1,000 x g, and collect the solution that contains the desired biotin-labelled protein.

Storage of Protein Conjugate

The antibody conjugate should be stored at > 0.5 mg/mL in the presence of a carrier protein (e.g., 0.1% bovine serum albumin). The conjugate solution could be stored at 4 °C for two months without significant change when stored in the presence of 2 mM sodium azide and kept from light. For longer storage, the protein conjugates could be lyophilized or divided into single-used aliquotes and stored at ≤ –60 °C.

Centrifugation Notes

Spin column (Component C) can fit into 2 mL microcentrifuge tubes or 12 x 75 mm test tubes for sample collection during centrifugation. Use the 2 mL microtube with the columns for the initial column equilibration step. Swinging bucket centrifuges capable of generating a minimum force of 1,000 x g are suitable for Bio-Spin column use. The gravitational force created at a particular revolution speed is a function of the radius of the microcentrifuge rotor. Consult the swinging bucket centrifuge instruction manual for the information about conversion from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, use the following equation to calculate the speed in RPM required to reach the gravitational force of 1,000 x g. RCF (x g) = (1.12 x 10 -5)×(RPM)×2×r (RCF is the relative centrifugal force, r is the radius in centimeters measured from the center of the rotor to the middle of the Bio-Spin column, and RPM is the speed of the rotor).

EXAMPLE DATA ANALYSIS AND FIGURES

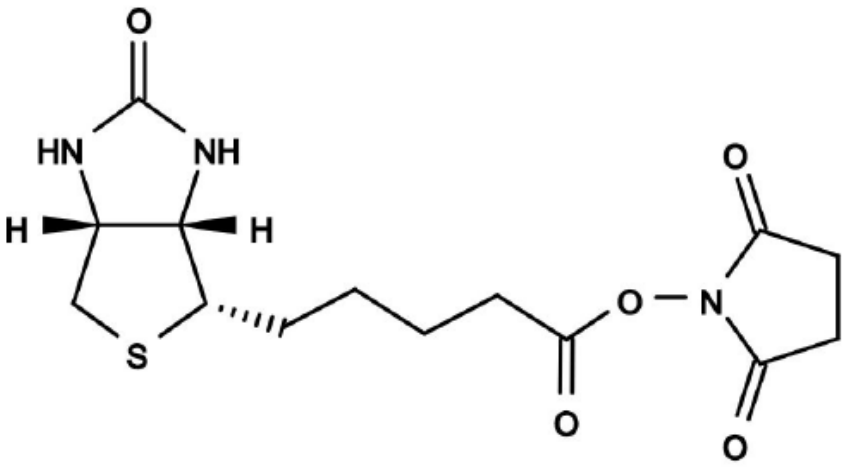

Figure 1. Chemical structure for Protein Biotinylation Kit.