ReadiLink™ Protein Biotinylation Kit *Powered by ReadiView™ Biotin Visionization Technology*

Catalog number: 5521
Unit size: 3 Reactions

Component	Storage	Amount
Component A: ReadiView™ Biotin SE	Freeze (< -15 °C)	1 vial
Component B: Reaction Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 vial (200 µL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (100 µL)
Component D: Spin Column	Room temperature (10-25 °C)	3 columns
Component E: Washing Tube (2 mL)	Room temperature (10-25 °C)	3 tubes
Component F: Collecting Tube (1.5 mL)	Room temperature (10-25 °C)	3 tubes

OVERVIEW

Biotin is widely used for labeling biomolecules, in particular, antibodies. This kit is primarily used for the preparation of biotin-labeled IgG for enzyme immunoassay (EIA). Our kit uses our ReadiView™ biotin succinimidyl ester (#3059) that reacts with an amino group of IgG and other biomolecules. Our unique biotin contained in the kit carries a color tag for indicating the degree of biotinylation, thus eliminating the troublesome HABA biotinylation determination. This kit contains all of the necessary reagents for labeling and purification. On our hands, 5 to 8 biotin molecules can be conjugated to each IgG molecule using our kit.

AT A GLANCE

Protocol Summary

1. Add 5 µL Reaction Buffer (Component B) into target protein (50 µL)
2. Add the protein solution into ReadiView™ Biotin SE (Component A)
3. Incubate at room temperature for 30-60 minutes
4. Purify the conjugate by spin column

Important Upon receipt, store ReadiView™ Biotin SE (Component A) at -20 °C, kept from moisture. Store other components at room temperature. Do not freeze Reaction Buffer (Component B) and Spin Column (Component D). 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 STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

ReadiView™ Biotin SE stock solution
Add 10 µL of DMSO (Component C) into the vial of ReadiView™ Biotin SE (Component A), and vortex them vigorously.

Note Prepare the ReadiView™ Biotin SE stock solution (Solution B) before starting the conjugation. Use promptly. Extended storage of the ReadiView™ Biotin SE stock solution may reduce the biotin activity. Solution B can be stored in freezer for two weeks when kept from moisture.

Note Aliquot the ReadiView™ Biotin SE stock solution into 5 vials (2 µL/vial). ONLY one vial is needed for labeling 100 µg proteins. The remaining vials can be stored in freezer for 2 weeks in case you need to repeat your conjugation.

PREPARATION OF WORKING SOLUTION

Protein working solution
For labeling 100 µg target protein (assuming the target protein concentration is 2 mg/mL), mix 5 µL (10% of the total reaction volume) of Reaction Buffer (Component B) with 50 µL of the target protein solution.

Note If you have a different protein concentration, adjust the protein volume accordingly to make ~2 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 ReadiView™ Biotin SE stock solution (2 µL/vial), and mix them well by repeatedly pipetting for a few times or vortex the vial for 2-5 minutes.

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 D) 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, Component E). 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, Component F). Carefully load the sample (20-100 µL, from Step conjugation reaction) directly to the center of the column.

2. After loading the sample, add 1X PBS (pH 7.2-7.4) to make the total volume of 110 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 D) 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) = $(1.12 \times 10^{-5})\times(RPM)\times 2\times r$ (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).

Degree of Biotinylation

The degree of biotinylation can be readily calculated by the following equation with a simple absorption spectrum:

Number of Biotin/Conjugate = $[A_{360}/9900] \div [A_{280}/\varepsilon_{protein}]$

A_{360} and A_{280} are the absorbances of the conjugation at 360 and 280 nm respectively, and $\varepsilon_{protein}$ is the extinction coefficient of the antibody or protein to be labeled.

EXAMPLE DATA ANALYSIS AND FIGURES

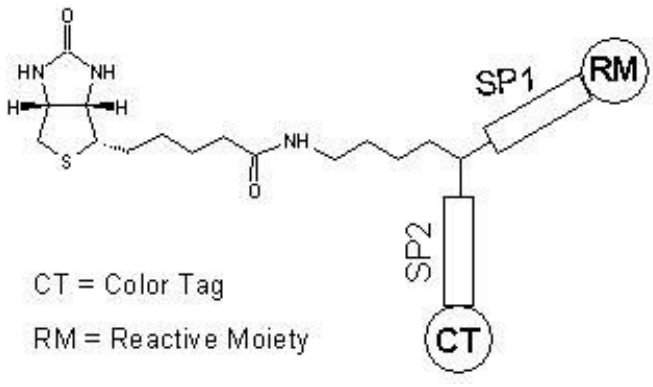

Figure 1.
ReadiView™ biotin conjugated with specially designed color tag (CT) for easy determination of biotinylation degree.

DISCLAIMER

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