# ReadiLink™ Protein Biotinylation Kit

**Powered by ReadiView™ Biotin Visionization Technology**

| Ordering Information | Storage Conditions |
|---|---|
| Product Number: 5521 (3 reactions) | Multiple storage conditions required |

## Introduction

Biotin is widely used for labeling biomolecules, in particular, antibodies. This ReadiLink™ Protein Biotinylation Kit is primarily optimized for the preparation of biotin-labeled IgG for enzyme immunoassay (EIA). It uses ReadiView™ biotin succinimidyl ester (Cat. #3059) that reacts with the amino groups of IgG and other biomolecules. Our unique ReadiView™ biotin SE carries a color tag to indicate the degree of biotinylation, thus eliminating the troublesome HABA biotinylation determination step. The HABA biotinylation assay is notoriously inaccurate although many efforts have been taken to improve the accuracy (including our kit 5522). The color tag is carefully selected to avoid the interference of either biotin binding or fluorescence detection. This kit comes with all the necessary reagents for labeling and purification. On our hands, 3 to 10 biotin molecules can be conjugated to each IgG molecule using this kit. The kit is designed for 3 conjugation reactions. For each conjugation reaction the material can label up to 100 µg protein. The entire process only takes less than an hour. 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.

## Kit Components

| Components | Amount | Storage |
|---|---|---|
| Component A: ReadiView™ Biotin SE | 1 vial | -20 °C |
| Component B: Reaction Buffer | 1 vial (200 µL) | Do not freeze |
| Component C: DMSO | 1 vial (100 µL) | -20 °C to room temperature |
| Component D: Spin Column | 3 columns | Do not freeze |
| Component E: Washing Tube (2 mL) | 3 tubes | Do not freeze |
| Component F: Collecting Tube (1.5 mL) | 3tubes | Do not freeze |

## Storage and Handling

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).*

## Standard Operating Protocol

*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.*

**1. Prepare protein solution (Solution P):**

Assuming the concentration of the target protein solution (antibody solution) 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. If you have a difference protein concentration, adjust the protein volume accordingly to make ~100 µg protein available for this labeling reaction

**Note 1**: *The pH of the protein solution (Solution P) 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 2**: *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 3**: *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 4**: *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.*

**2. Prepare ReadiView™ Biotin SE stock solution (Solution B):**

Add 10 µL of DMSO (Component C) into the vial of ReadiView™ Biotin SE (Component A), and vortex the vial vigorously.
**Note 1:** *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 2:** *Aliquot the ReadiView™ Biotin SE stock solution into 5 vials (2 µL/vial). ONLY one vial is needed for labeling 100 ug proteins. The remaining vials can be stored in freezer for 2 weeks in case you need repeat your conjugation.*

**3. Run conjugation reaction:**

3.1 Add the protein solution (Solution P) into the vial of ReadiView™ Biotin SE stock solution (2 µL/vial, Solution B), and mix them well by repeatedly pipetting for 2-5 minutes.
**Note:** *The DMSO concentration should be kept less than 10%.*

3.2 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.*

**4. Prepare spin column for sample purification:**

4.1 Invert the Spin Column (Component D) sharply several times to resuspend the settled gel and remove any bubbles.

4.2 Snap off the tip and place column in a Washing Tube (2 mL, Component E). Remove the cap to allow the excess packing buffer to drain by gravity to the surface 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.

4.3 Centrifuge for 1 min in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.

4.4 Apply 2 mL 1X PBS, pH 7.2 -7.4 into the column. After each application of PBS, let the buffer drain by gravity, or centrifuge the column for 1 min to remove the buffer. Discard buffer from collection tube. Repeat for 3-4 times.

4.5 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.

**5. Purify the conjugation:**

5.1 Place the column (from Step 4.5) in a clean Collecting Tube (1.5 mL, Component F). Carefully load the sample (20 −100 µL) directly to the center of the column.

5.2 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 minutes at 1,000 x g, and collect the solution that contains the desired dye-labeled protein.
**Note 1:** *For immediate use, the dye-protein conjugate need be diluted with staining buffer, and aliquoted for multiple uses.*
**Note 2:** *For longer term storage, the dye-protein conjugate solution need be concentrated or freeze dried (see below).*

## Storage of Protein Conjugate

The protein 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 aliquots 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 microtubes provided 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) = $(1.12 \times 10^{-5})\times(\text{RPM})\times 2\times r$ (*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*).

## References

1. Hirsch JD, Haugland RP (2005). Conjugation of antibodies to biotin. Methods Mol Biol, 295, 135.
3. Haugland RP, You WW (1998). Coupling of antibodies with biotin. Methods Mol Biol, 80, 173.
3. Haugland RP, You WW (1995). Coupling of monoclonal antibodies with biotin. Methods Mol Biol, 45, 223.