ReadiView™ biotin succinimidyl ester

Catalog number: 3059
Unit size: 5 mg

Component	Storage	Amount (Cat No. 3059)
ReadiView™ biotin succinimidyl ester	Freeze (< -15 °C)	1 vial (5 mg)

OVERVIEW

Biotin/avidin complexes are widely applied for a variety of biological detections. Although a large number of biotin-labeled bioconjugates are commercially available, the accurate determination of biotinylation degree (ratio of biotin/biopolymer) is still a great challenge for biochemists. HABA is still predominantly used for determining the degree of biotinylation (through its absorption with the extinction coefficient = 34,000/M-1cm-1). When a biotin-containing sample is added, the biotin binds strongly to avidin and displaces the weakly bound HABA. The resulting decrease in absorbance relates to the amount of biotin. However there are many factors that affect the accuracy of HABA method, making this method unreliable for many biotin-labeled conjugates. Our ReadiView™ biotin contains specially designed Color Tag (CT) that makes the biotinylation degree readily accessible by simply calculating the corrected absorption ratio of 280 nm/385 nm. Our specially designed tag has very minimal effect on the biotin binding affinity, and its absorption maximum was designed to make the tag have minimal quenching effect on the most fluorophores that are used for labeling avidins.

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

Protein stock solution (Solution A)

Mix 100 µL of a reaction buffer (e.g., 1 M sodium carbonate solution or 1 M phosphate buffer with pH ~9.0) with 900 µL of the target protein solution (e.g. antibody, protein concentration >2 mg/mL if possible) to give 1 mL protein labeling stock solution.

Note: The pH of the protein solution (Solution A) 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 1 M sodium bicarbonate solution or 1 M pH 9.0 phosphate buffer.

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 will 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.

ReadiView™ biotin succinimidyl ester stock solution (Solution B)

Add anhydrous DMSO into the vial of ReadiView™ biotin succinimidyl ester to make a 10 mM stock solution. Mix well by pipetting or vortex.

Note: Prepare the ReadiView™ biotin succinimidyl ester (Solution B) before starting the conjugation. Use promptly. Extended storage of the ReadiView™ biotin succinimidyl ester stock solution may reduce its activity. Solution B can be stored in freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with ReadiView™ biotin succinimidyl ester. You might need further optimization for your particular proteins.

Note: Each protein requires distinct ReadiView™ biotin succinimidyl ester/protein ratio, which also depends on the properties of ReadiView™ biotin succinimidyl ester. Over labeling of a protein could detrimentally affects its binding affinity while the protein conjugates of low ReadiView™ biotin succinimidyl ester/protein ratio gives reduced sensitivity.

Run conjugation reaction

1. Use 10:1 molar ratio of Solution B (ReadiView™ biotin succinimidyl ester)/Solution A (protein) as the starting point: Add 5 µL of the ReadiView™ biotin succinimidyl ester stock solution (Solution B, assuming the ReadiView™ biotin succinimidyl ester stock solution is 10 mM) into the vial of the protein solution (95 µL of Solution A) with effective shaking. The concentration of the protein is ~0.05 mM assuming the protein concentration is 10 mg/mL and the molecular weight of the protein is ~200KD.

   Note: We recommend to use 10:1 molar ratio of Solution B (ReadiView™ biotin succinimidyl ester)/Solution A (protein). If it is too less or too high, determine the optimal ReadiView™ biotin succinimidyl ester/protein ratio at 5:1, 15:1 and 20:1 respectively.

2. Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

Purify the conjugation

The following protocol is an example of dye-protein conjugate purification by using a Sephadex G-25 column.

1. Prepare Sephadex G-25 column according to the manufacture instruction.
2. Load the reaction mixture (From "Run conjugation reaction") to the top of the Sephadex G-25 column.
3. Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top resin surface.
4. Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification. Combine the fractions that contain the desired ReadiView™ biotin-protein conjugate.

   Note: For immediate use, the ReadiView™ biotin-protein conjugate need be diluted with staining buffer, and aliquoted for multiple uses.

   Note: For longer term storage, ReadiView™ biotin-protein conjugate solution need be concentrated or freeze dried.

Characterize the Desired Biotin-Protein Conjugate

The Degree of Substitution (DOS) is the most important factor for characterizing Biotin-labeled protein. The Biotin DOS depends on the number of thiol groups on the protein. For effective labeling, the degree of substitution should be controlled to have 4-8 moles of Biotin to one mole of antibody.

Measure absorption

To measure the absorption spectrum of a Biotin-protein conjugate, it is recommended to keep the sample concentration in the range of 1- 10 μM depending on the extinction coefficient of the Biotin.

Read OD (absorbance) at 280 nm and biotin maximum absorption (λmax = 385 nm for biotin)

For most spectrophotometers, the sample (from the column fractions) needs to be diluted with de-ionized water so that the O.D. values are in the range of 0.1 to 0.9. The O.D. (absorbance) at 280 nm is the maximum absorption of protein, while 385 nm is the maximum absorption of biotin. To obtain accurate DOS, ensure the conjugate is free of the non-conjugated biotin.

Calculate DOS

You can calculate DOS using our tool by following this link: https://www.aatbio.com/tools/degree-of-labeling-calculator

EXAMPLE DATA ANALYSIS AND FIGURES

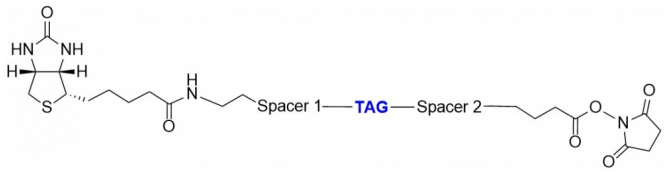

Figure 1. The structure of ReadiView™ biotin succinimidyl ester that is imbedded with a conjugation TAG. This imbedded TAT can be easily monitored by its UV absorption distinct from the 280 nm absorption of proteins. The TAG does not quench fluorescence of the commonly used fluorescent dyes with the minimal effect on avidin binding.

DISCLAIMER

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