

ReadiView™ biotin maleimide

Catalog number: 3058
Unit size: 5 mg

Component	Storage	Amount (Cat No. 3058)
ReadiView™ biotin maleimide	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.

SAMPLE EXPERIMENTAL PROTOCOL

Labeling Proteins with ReadiView™ biotin maleimide

1. Dissolve your thiol-containing protein at concentration of 1 - 10 mg/mL (3 - 10 mg is the optimal labeling concentration) using PBS buffer (20 mM, pH 7.2).
2. Dissolve the ReadiView™ biotin maleimide in DMSO at a concentration of 5 - 10 mg/mL.
3. Mix the ReadiView™ biotin maleimide and protein solution at appropriate molar ratio of biotin/protein (use 10:1 as a starting point and optimize accordingly), and shake the reaction mixture at room temperature for 2 - 4 hours.
4. Filter the reaction mixture through a protein spin column for 100 µg to 1 mg protein labeling reaction. If the reaction scale is larger than 1 mg, purify the conjugate using gel filtration on a properly sized Sephadex G-25 column.
5. Collect the desired fractions for your immediate use or freeze dry them for future use.

Labeling Small Molecules with ReadiView™ biotin maleimide

1. Dissolve ReadiView™ biotin maleimide (10 - 15 mg/mL) and your thiol-containing molecule in DMSO at 1:1.2 molar ratio of biotin/thiol-containing molecule.
2. Stir the reaction mixture at room temperature for 2 - 4 hours.
3. Purify the conjugate using HPLC (ammonium acetate/water and acetonitrile, pH 7.0).
4. Collect and pool the desired fractions.
5. Combine and freeze-dry the pooled fractions.

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 ($\lambda_{max} = 385$ 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

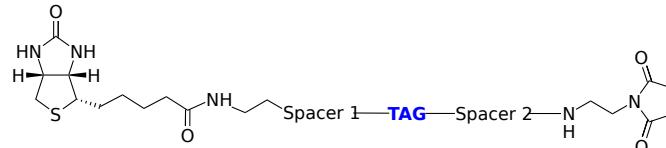


Figure 1. Chemical structure for ReadiView™ biotin maleimide

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