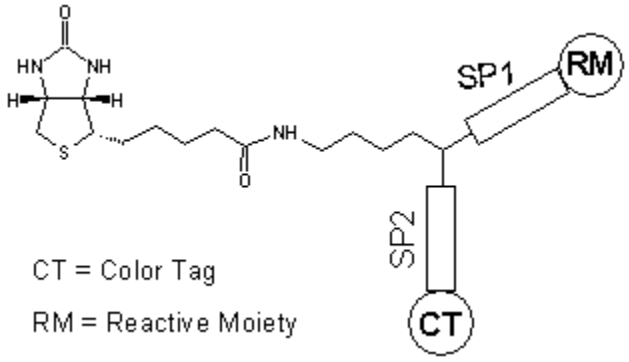
Biotinylation of Antibodies with ReadiView™ Biotin Probes

Introduction

Biotin/avidin complexes are widely applied for a variety of biological detections. Biotin is used in two-step detection systems in concert with conjugated avidin. It is typically conjugated to proteins via primary amines (i.e., lysines). Usually, between 3 and 6 biotin molecules are conjugated to each antibody. Although a large number of biotin-labeled bioconjugates are commercially available, the accurate determination of biotinylation degree (ratio of biotin/biopolymer) remains 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 it 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 most of the fluorophores that are used for labeling avidins.



Conjugation Protocol

1. Prepare antibody solution:

1.1 Dialyze or exchange over a column the antibody in "Reaction Buffer".
Note: It is critical that sodium azide be completely removed from any antibody.

1.2 Measure the antibody concentration after buffer equilibration. If necessary, dilute the antibody to a concentration of 2 to 10 mg/mL.
Note: For effective labeling, an antibody concentration of at least 2 mg/mL is recommended. The extent of biotin conjugation to the antibody may depend on the concentration of antibody in solution. For consistent conjugations, a consistent concentration is recommended.

2. Run conjugation:

2.1 Dissolve 10 mg of a biotin SE in 1 mL anhydrous DMSO immediately before use.
Note: The reactive biotin molecule is unstable. Once the biotin is dissolved, it should be used immediately.

2.2 Add biotin to give a ratio of 0.1 mg per mg of antibody, and mix immediately.
Note: When first conjugating an antibody, a range of biotin to antibody concentrations should be compared, e.g., a range of 0.05 to 0.5mg biotin per mg of antibody (for instance, 10, 50, 100, 200, 500, 800 µg per mg). Compare each conjugate by your desired staining method and select the conjugate with the brightest "positive" cells which have low background on "negative" cells.
2.3 Wrap the tube in foil; incubate and rotate at room temperature for 2-4 hours.

2.4 Remove the unreacted biotin by column separation.

2.5 Exchange the antibody into "Storage Buffer" by gel filtration or dialysis.

Appendix: Materials, Chemicals and Buffers Used for Biotinylation

1. Column separations:

For 1.25 mL to 2.5 mL sample volumes: PD-10 (Sephadex G-25M), Amersham, **Cat. #** 17-0851-01.
For <0.5 mL sample volumes: NAP5 columns (Sephadex G-25 DNA grade), Amersham**, Cat.** # 17-0853-02.

2. Chemicals:

DMSO - anyhydrous dimethyl sulfoxide
Note: Keep the DMSO absolutely dry at all times. Store in a desiccator. Use only sufficient amount of DMSO.

3. Reaction buffers:

100 mM carbonate, pH 8.4
Note: Sodium azide cannot be added into this buffer.

4. Storage buffer:

10 mM Tris, 150 mM NaCl, 0.1% NaN_3, pH 8.2