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INSTRUCTIONS

Antibody Biotinylation Kit

Product Number **D5014**
Store at RT

INTRODUCTION

Biotin is used in two-step detection systems in concert with conjugated avidin. Biotin is typically conjugated to proteins via primary amines (i.e., lysines). Usually, between 3 and 6 biotin molecules are conjugated to each antibody.

The entire conjugation can be performed in about an hour. In general, you will need to have a solution of your antibody at a concentration (optimally) of at least 2 mg/ml. The extent of biotin conjugation to the antibody may depend on the concentration of antibody in solution; for consistent conjugations, use a consistent concentration. This product provides convenient and efficient method for removing salt and amine compound interfering biotin coupling reaction from antibody solution.

Kit contents

	10 reactions
1M Sodium Acetate pH4.0	1ml x 1
Carprylic Acid	0.5ml x 1
Neutralizing Buffer	1ml x 1
Solution A	8ml x 1
Labeling Buffer	8ml x 1
DMSO	0.1ml x 1
Reactive Biotin	1
Stop Solution	0.3ml x 1
Solution B	8ml x 1

Additional Materials Required

- Ultrapure water
- 80% Glycerol (Optional)

Alternatives For Reactive Biotin

Reactive Biotin, NHS-Biotin, could be replaced by commercially available activated biotin,

Procedure Summary

1. Antibody Purification
2. Desalting and Buffer Exchange
3. Biotin labeling
4. Stopping and Storage

Procedure

Antibody Purification by Carprylic Acid precipitation

1. Add 500μl of DW to 500μl serum and acidify by adding 60μl of 1M sodium acetate pH 4.0

2. Slowly (drop-wise) add 37μl (20ul for rat or mouse serum) of carprylic acid and continue stirring for 20 min at room temperature.

3. Centrifuge at 12,000xg for 10min. and carefully remove and save the supernatant.

4. Adjust the pH 7.5~8.5 of supernatant antibody solution by adding 5μl neutralizing solution per 100μl supernatant solution and if necessary, centrifuge the solution at 8,000xg for 5min. and discard precipitate.

Alternatively start here if you have your own affinity purified IgG.

Desalting and Buffer Exchange

5. Add half volume of Solution A (0.5ml) and mix thoroughly by gentle inverting then stand for 10 min

6. Precipitate the immunoglobulin aggregate by centrifugation at 12,000xg for 5min and remove completely the turbid supernatant.(If necessary, recentrifuge the pellet briefly for 20 second to remove the residual turbid solution)

7. Dissolve the precipitated immunoglobulin with 300μl Labeling Buffer (to be approximate 2mg/ml IgG).

8. If there is any insoluble material discard it by high speed centrifugation for 10 min.

Biotin Labeling

9. Dissolve the Reactive Biotin with 60 μl DMSO(**10mM in DMSO**). Aliquote and store at -70°C. Add 2μl Reactive Biotin solution per 100μl IgG solution and incubate 30min at room temperature.

10. At the end of the incubation, add 2μl Stop Solution/100μl solution and subsequently add half volume of Solution B to aggregate the biotin labeled IgG and remove the residual reactive biotin. Stand this suspension at 4°C for 20min.

11. Centrifugation the suspension at 12,000xg for 10min and dissolve the aggregated IgG with equal volume of Labeling Buffer(phosphate buffer). Add 80% glycerol and store at -20℃.