



APPLICATION

- Immunohistochemical staining
- Flow Cytometry
- Radio-, enzyme-, and fluorescent immunoassays

REAGENTS

1. 0,1 M Na-carbonate buffer pH: 9.5, without NaN_3
2. NHS-Biotin
3. Dimethyl Sulfoxide (DMSO)
4. 0,1 M Phosphate Buffered Saline (PBS) pH: 7.0-7.2.
5. Thimerosal

Note: It is critical that sodium azide (NaN_3) be completely removed from any antibody and solution.

PREPARATION OF REAGENTS

Dissolve NHS-Biotin in DMSO to a 1 mg/ml solution immediately before use.

PREPARATION OF ANTIBODY

1. Dialysis of the chicken antibodies against 0,1 M Na-Carbonate Buffer pH: 9.5.

Dialysis of the chicken antibodies in 250 ml Na-Carbonate Buffer pH: 9.5 at room temperature for at least 2 hours. Change the Na-Carbonate Buffer pH: 9.5 at least 4 times.

2. Use chicken antibodies (IgY) with a concentration of at least 1 mg/ml.

Determine the IgY concentration of the dialyzed chicken antibody solution. Dilute the chicken antibodies 1:10 in Na-Carbonate Buffer pH: 9.5 and measure the concentration with a spectrophotometer at an optical density of 280 nm (OD_{280}). Calculate the IgY concentration according the following:

$$\text{IgY concentration (mg/ml)} = \text{OD}_{280} \text{ value} \times 10 / 1.36$$

If the antibody concentration is less than 1 mg/ml, the conjugation will probably be sub-optimal.



COVALENT CONJUGATION

1. Starting the biotinylation process

Add NHS-Biotin in DMSO to chicken antibody at desired ratio of 4:1, e.g. add 0.25 ml of NHS-Biotin to 1ml of chicken antibody.

Stir for 3 minutes at room temperature.

Incubate for 1-2 hours at room temperature.

2. Dialyzation

Dialysis of the biotinylated chicken antibody solution in 250 ml PBS at room temperature for at least 2 hours. Change the PBS at least 4 times.

3. Storage

Add v/v 0.01% thimerosal to the dialyzed biotinylated chicken antibody solution.

Store at +4 °C.