



## Protocol for Biotinylation of Chicken Antibodies

Protocol for Biotinylation of IgY/version 2.0

### APPLICATION

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

### REAGENTS

1. 0.1 M Sodium carbonate buffer pH: 9.5, without  $\text{NaN}_3$
2. NHS-Biotin
3. Dimethyl Sulfoxide (DMSO)
4. 0.01 M Phosphate Buffered Saline (PBS) pH: 7.2.
5. Thimerosal or Sodium-azide solution

Note: It is critical that sodium azide ( $\text{NaN}_3$ ) be completely removed from the antibody solution before the starting the biotinylation process.

### 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 ( $\text{OD}_{280}$ ). 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.



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### COVALENT CONJUGATION

#### 3. 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.

#### 4. 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.

#### 5. Storage

Add thimerosal or sodium azide to the dialyzed biotinylated chicken antibody solution. Add thimerosal or sodium azide to a final concentration of 0.02% (v/v). Store at +4 °C.