



Protocol for Horseradish Peroxidase (HRP) Conjugation of Chicken Antibodies(1 mg/ml)

Protocol for HRP conjugation of IgY/version 1.0

APPLICATION

- Enzyme immunoassays
- Western blot applications
- Tissue staining

REAGENTS

1. Horseradish peroxidase (HRP)
2. Chicken antibodies (IgY)
3. Glutaraldehyde 25 % solution in water
4. 1M Tris pH:7,2
5. 0,1 M Phosphate Buffered Saline (PBS) pH: 7.0-7.2.
6. Glycerol.

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

PREPARATION OF REAGENTS

Glutaraldehyde 1 % solution in PBS

Add 40 μl Glutaraldehyde 25 % solution to 960 μl PBS for each ml working solution.

PREPARATION OF ANTIBODY

Determine the IgY concentration if it is unknown

Dilute the chicken antibodies 1:10 in PBS 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 2 mg/ml, the conjugation will probably be sub-optimal.



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HRP CONJUGATION

- 1. Add 2 mg/ml HRP to the chicken antibodies (conc. 2 mg/mg).**
Stir for 3 minutes at room temperature.
- 2. Activation of the chicken antibody-HRP solution.**
Add 80 μ l per ml chicken antibody-HRP solution.
Stir for 3 minutes at room temperature.
Incubate for 2.5 to 3 hours at room temperature.
- 3. Stopping the activation process.**
Add 108 μ l 1M Tris pH:7,2 per ml activated chicken antibody-HRP solution.
Stir for 3 minutes at room temperature.
Incubate for 1 hour at room temperature.
- 4. Dialyzation**
Dialysis of the processed chicken antibody-HRP solution in 250 ml PBS at room temperature for at least 2 hours. Change the PBS at least 4 times.
- 5. Storage**
Add 50% Glycerol to the dialyzed chicken antibody-HRP solution.
Store at -20°C.