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

APPLICATION

- Enzyme immunoassays
- Western blot applications
- Tissue staining

REAGENTS

1. Horseradish peroxidase (HRP)
2. Chicken antibodies (IgY)
3. Glutaraldehyde 25% solution in water
4. 1.0 M Tris pH:7,2
5. 0.01 M Phosphate Buffered Saline (PBS) pH: 7.2.
6. Glycerol or sodium azide solution.

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

1% glutaraldehyde; For each ml needed, add 40 µl glutaraldehyde (25%) solution to 960 µl PBS. 1 ml of 1% glutaraldehyde will be enough for activation of 12.5 ml of chicken antibody-HRP solution.

PREPARATION OF ANTIBODY

1. 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:

IgY concentration (mg/ml) = OD_{280} value x 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

2. **Add 2 mg/ml HRP to the chicken antibodies (conc. 2 mg/mg).**
   Stir for 3 minutes at room temperature.

3. **Activation of the chicken antibody-HRP solution.**
   Add 80 µl 1% glutaraldehyde per ml chicken antibody-HRP solution.
   Stir for 3 minutes at room temperature.
   Incubate for 2.5 to 3 hours at room temperature.

4. **Stopping the activation process.**
   Add 108 µl 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.

5. **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.

6. **Preservation and storage**
   Add 50% Glycerol to the dialyzed chicken antibody-HRP solution.
   Store at -20°C.

   ALTERNATIVELY:

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