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. 1M Tris pH:7.2
5. 0.1 M Phosphate Buffered Saline (PBS) pH: 7.0-7.2.

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:

\[
\text{IgY concentration (mg/ml)} = \frac{\text{OD}_{280} \times 10}{1.36}
\]

If the antibody concentration is less than 2 mg/ml, the conjugation will probably be sub-optimal.
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 µl 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 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.

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.