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# Preparation of horseradish peroxidase (HRP) conjugated to chicken anti-IgY

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## ABSTRACT

Avian IgY is structurally and genetically different from its mammalian counterpart IgG. This reagent can be used in ELISA, Western blotting and Dot blot to detect specific chicken IgY to infectious agents or immune system proteins [1]. IgY can be isolated by the Polson Method [2] or the water dilution method [3]. Chicken IgY can cross-react with IgY from diverse bird species [4]. Similarly avian IgY can be isolated from their egg yolk using the same methodology with modifications that was developed for the chicken IgY isolation [5].

## Reference

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## CREATED

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## GUIDELINES

All reagents but specially the enzyme and sodium periodate solution has to be prepared freshly before mixing it with the enzyme. Chicken anti-IgY can be prepared by immunizing an animal species with purified chicken IgY; which can be isolated by a protein purification method and refined by affinity chromatography. The high lipid content of the egg yolk can be separated from the water soluble fraction where is the IgY by the use of chloroform.

## MATERIALS

NAME	CATALOG #	VENDOR
Ammonium Sulfate		P212121
Sodium periodate	SB0875.SIZE.100g	Bio Basic Inc.
sodium borohydride	452882	Sigma Aldrich
AF647 Donkey anti-chicken IgY (IgG)	703-605-155	Jackson ImmunoResearch
Horseradish Peroxidase (HRP) type IV	P8375-25KU	Sigma Aldrich
Pierce&trade; Chicken IgY Purification Kit	44922	Thermo Fisher

- 1 Horseradish peroxidase (500 µg in 50 µl NaCO<sub>3</sub>, pH 9.6) is mixed with freshly made sodium periodate solution (1.71 mg/ml) followed by incubation in the dark for 2 h.
- 2 Mix 500µg of chicken anti-immunoglobulin Y (anti-IgY) developed in a laboratory animal or commercially available with 500 µg of the mix of horseradish peroxidase-sodium periodate that was prepared above.
- 3 The mixture is incubated for 3 hours at 4°C with gentle agitation.
- 4 Forty µl of freshly prepared NaBH<sub>4</sub> solution (5 mg NaBH<sub>4</sub> /ml 0.1 mM NaOH) is then added to the preparation.
- 5 The preparation is incubated for 90 min at 4°C in the dark with gentle agitation.
- 6 Cold 50% saturated ammonium sulphate solution (pH 7.4) is added drop by drop in the ratio 1:1 (v/v).
- 7 The mixture is then centrifuged for 25 min at 4°C and recover the pellet at the bottom of the tube.
- 8 The pellets is re-suspended in 200 µl of PBS pH=7.4 and dialysed against 1L of PBS for 24 h with 3 buffer changes.

- 9 An equal volume of glycerol is added to the dialysate followed by 100 µl of bovine serum albumin, BSA (20 mg/ ml).
- 10 The peroxidase conjugated to anti-chicken IgY is then stored at -20°C until further used.