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Direct ELISA for investigating the binding of peroxidase-labeled anti-chicken IgY conjugate with avian immunoglobulins

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1 Works for me dx.doi.org/10.17504/protocols.io.bjxykppw

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ABSTRACT

The peroxidase-labeled anti-chicken IgY conjugate cross-reacts with many IgY present in the egg of many and diverse avian species.

References

1. Justiz Vaillant AA, Ramirez N, Cadiz A, Ferrer B, Akpaka P, et al. (2013) Separation and Reactivity of Avian Immunoglobulin Y. J Chromat Separation Techniq 4: 173. doi:10.4172/2157-7064.1000173

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MATERIALS

NAME	CATALOG #	VENDOR
Anti-Chicken IgY, HRP Conjugate, 300ul	G1351	Promega
Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, V 96 well plate, Non-Treated, clear, without lid, Sterile	260210	Thermo Fisher

1 This ELISA is used to study the interaction of anti-chicken IgY-HRP conjugate with diverse avian immunoglobulins.

2 The 96 well microtitre plate is coated overnight at 4°C with 1 µg/µl per well of purified avian immunoglobulins or 50 µl of water soluble fraction from egg yolks of avian species in carbonate-bicarbonate buffer pH 9.6.

3 Then plate is treated with bovine serum albumin solution and washed 4X with PBS-Tween.

4 Then 50 µl of peroxidase-labeled-anti-chicken IgY conjugate diluted 1:15000 in PBS-non-fat milk is added to each well and incubated for 1.30h at RT. After that the plate is washed 4X with PBS-Tween.

5 Pipette 50 µl of 3,3',5,5' - tetramethylbenzidine (TMB; Sigma-Aldrich) to each well.

6 The reaction is stopped with 50 µl of 3M H_2SO_4 solution.

7 The plate is visually assessed for the development of colour and read in a microplate reader at 450 nm.

8 A cut-off point should be calculated as the mean of the optical density of negative controls x 2 SD.

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