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

Angel A Justiz-Vaillant

1University of the West Indies St. Augustine

University of the West Indies  angel.vaillant@sta.uwi.edu

ABSTRACT

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

References


MATERIALS

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

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Protocol status: Working
We use this protocol and it's working

Created: Aug 19, 2020
This ELISA is used to study the interaction of anti-chicken IgY-HRP conjugate with diverse avian immunoglobulins.

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.

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

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.

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

The reaction is stopped with 50 µl of 3M H2SO4 solution.

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

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