AUG 19, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bjxykppw

Protocol Citation: Angel A Justiz-Vaillant 2020. Direct ELISA for investigating the binding of peroxidase-labeled anti-chicken IgY conjugate with avian immunoglobulins.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.bjxykppw>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Aug 19, 2020

Last Modified: Aug 19, 2020

PROTOCOL integer ID:
 40664

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

Angel A Justiz-Vaillant¹

¹University of the West Indies St. Augustine

University of the West Indies

angel.vaillant@sta.uwi.edu

Angel A Justiz-Vaillant

University of the West Indies St. Augustine

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

MATERIALS

MATERIALS

⊗ 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**

- 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 H₂SO₄ 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.