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Direct ELISA for investigating the binding of chemically-made Protein-LAG-anti-IgY-peroxidase to both avian and mammalian immunoglobulins.

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We use this protocol and it's working

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Abstract

Peroxidase-labeled-protein-LAG-anti-IgY conjugate is chemically made. It has unique binding properties. It binds to both avian and mammalian immunoglobulins. It can be used as a reagent in ELISA, Western blotting and dot blot for immunodetection of immunoglobulins and antigens. It may be used to make the immunodiagnosis of infectious diseases involving laboratory, wild, zoo, and farm animals [1].

References

1.Vaillant AJ, McFarlane-Andersonv N, Wisdom B, Mohammed W, Vuma S, et al. (2013) Immunoglobulin-binding Bacterial Proteins (IBP) Conjugates and their Reactivity with Immunoglobulin in Enzyme-Linked Immunosorbent Assays (ELISA). J Anal Bioanal Tech 4: 175. doi:10.4172/2155-9872.1000175

Materials

MATERIALS

- Anti-Chicken IgY, HRP Conjugate, 300ul **Promega Catalog #**G1351
- Horseradish peroxidase (HRP) **Gold Biotechnology Catalog #**P-100
- Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, V 96 well plate, Non-Treated, clear, without lid, Sterile **Thermo Fisher Catalog #**260210
- Staphylococcal Protein-A **Merck MilliporeSigma (Sigma-Aldrich)**
- Protein-L from P. Magnus
- Streptococcal protein G by Sigma Aldrich

Troubleshooting

1 This ELISA is used to study the interaction of protein-LAG-anti-IgY-HRP (SpLAG-anti-IgY-HRP) with diverse immunoglobulins.

2 The 96 well microtitre plate is coated overnight at 4°C with 1 µg/µl per well of purified immunoglobulins, 50 µl of any animal sera, or 50 µl of water soluble fraction from egg yolks 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-protein-LAG-anti-IgY conjugate diluted 1:5000 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.