Aug 19, 2020

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

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1 Works for me dx.doi.org/10.17504/protocols.io.bjxzkpp6

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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

DOI

dx.doi.org/10.17504/protocols.io.bjxzkpp6

PROTOCOL CITATION

Angel A Justiz-Vaillant 2020. Direct ELISA for investigating the binding of chemically-made Protein-LAG-anti-IgY-peroxidase to both avian and mammalian immunoglobulins.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjxzkpp6

CREATED

Aug 19, 2020

LAST MODIFIED

Aug 19, 2020

PROTOCOL INTEGER ID

40665

MATERIALS

NAME	CATALOG #	VENDOR
Anti-Chicken IgY, HRP Conjugate, 300ul	G1351	Promega
Horseradish peroxidase (HRP)	P-100	Gold Biotechnology

NAME	CATALOG #	VENDOR
Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, V 96 well plate, Non-Treated, clear, without lid, Sterile	260210	Thermo Fisher
Staphylococcal Protein-A		Sigma Aldrich
Protein-L from P. Magnus		
Streptococcal protein G by Sigma Aldrich		

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 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.

Citation: Angel A Justiz-Vaillant (08/19/2020). Direct ELISA for investigating the binding of chemically-made Protein-LAG-anti-IgY-peroxidaseÃÂ to both avian and mammalian immunoglobulins.. https://dx.doi.org/10.17504/protocols.io.bjxzkpp6