



Aug 18, 2020

Direct ELISA for investigating the binding of recombinant or chemically-made Protein-AG to immunoglobulins.

DOI

<https://dx.doi.org/10.17504/protocols.io.bjxwkppe>

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Protocol Citation: Angel Justiz-Vaillant 2020. Direct ELISA for investigating the binding of recombinant or chemically-made Protein-AG to immunoglobulins.. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bjxwkppe>

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Protocol status: Working

We use this protocol and it's working

Created: August 18, 2020

Last Modified: August 18, 2020

Protocol Integer ID: 40662

Keywords: mammalian immunoglobulin, fc regions of many mammalian immunoglobulin, immunoglobulin, protein ag, many mammalian immunoglobulin, linked immunosorbent assay, immunosorbent assay, bacterial protein, binding bacterial protein, reactivity with immunoglobulin, binding protein, direct elisa, protein, made protein, ag, enzyme, spag, elisa

Abstract

Protein AG (SpAG) is an immunoglobulin-binding protein that interacts with the Fc regions of many mammalian immunoglobulins [1].

References

1. Vaillant AJ, McFarlane-Anderson 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



Nunc®; 96-Well Polystyrene Round Bottom Microwell Plates, V 96 well plate, Non-Treated, clear, without lid, Sterile **Thermo Fisher Catalog #260210**



Protein-L from P. Magnus



Streptococcal protein G by Sigma Aldrich

- 1 This ELISA is used to study the interaction of protein-AG (SpAG) with diverse immunoglobulins.
- 2 The 96 well microtitre plate is coated overnight at 4°C with 1 µg/µl per well of purified immunoglobulins or 50 µl of any animal sera 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-AG conjugate diluted 1:3000 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.