Aug 19, 2020

Direct ELISA for investigating the binding of Protein-G to immunoglobulins.

DOI
dx.doi.org/10.17504/protocols.io.bjxrkpm6

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Protocol Citation: Angel A Justiz-Vaillant, Monica F. Smikle 2020. Direct ELISA for investigating the binding of Protein-G to immunoglobulins.. **protocols.io https://dx.doi.org/10.17504/protocols.io.bjxrkpm6**

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

Created: August 19, 2020

Last Modified: August 19, 2020

Protocol Integer ID: 40657

Keywords: streptococcal protein, mammalian immunoglobulin, binding bacterial protein, immunoglobulin, bacterial protein, fc region of many mammalian immunoglobulin, many mammalian immunoglobulin, linked immunosorbent assay, reactivity with immunoglobulin, binding of protein, immunosorbent assay, binding protein, protein, direct elisa

Abstract

Streptococcal protein G is an immunoglobulin-binding protein that interacts with the Fc region of many mammalian immunoglobulins [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

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

Streptococcal protein G by Sigma Aldrich

Troubleshooting

1 This ELISA is used to study the interaction of Streptococcal protein-G (SpG) 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-G 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.