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

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

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Abstract

Protein LG (SpLG) is an immunoglobulin-binding protein that interacts with the Fab and Fc regions 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
- Protein-L from P. Magnus
- Streptococcal protein G by Sigma Aldrich

Troubleshooting

1 This ELISA is used to study the interaction of protein-LG (SpLG) with diverse immunoglobulins. or chemically

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-LG 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 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.