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## Direct ELISA for investigating the binding of recombinant Protein-LA to immunoglobulins.

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**We use this protocol and it's working**

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## Abstract

Referencesst protein LA (SpLA) 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&trade; 96-Well Polystyrene Round Bottom Microwell Plates, V 96 well plate, Non-Treated, clear, without lid, Sterile **Thermo Fisher Catalog #260210**



Recombinant protein LA by Sigma-Aldrich

## Troubleshooting

- 1 This ELISA is used to study the interaction of recombinant protein-LA (SpLA) 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-LA 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<sub>2</sub>SO<sub>4</sub> 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.