



Aug 19, 2020

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

Angel A Justiz-Vaillant¹, Monica F. Smikle²¹University of the West Indies St. Augustine; ²University of the West Indies. Mona Campus**1** Works for me dx.doi.org/10.17504/protocols.io.bjxukpnw[University of the West Indies](#) angel.vaillant@sta.uwi.eduAngel Justiz-Vaillant
University of the West Indies St. Augustine

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-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

DOI

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

PROTOCOL CITATION

Angel A Justiz-Vaillant, Monica F. Smikle 2020. Direct ELISA for investigating the binding of recombinant Protein-LA to immunoglobulins.. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bjxukpnw>

LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Aug 19, 2020

LAST MODIFIED

Aug 19, 2020

PROTOCOL INTEGER ID

40660

MATERIALS

NAME	CATALOG #	VENDOR
Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, V 96 well plate, Non-Treated, clear, without lid, Sterile	260210	Thermo Fisher
Recombinant protein LA by Sigma-Aldrich		

- 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 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.