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Copy of Direct ELISA for investigating the binding of chemically-made Protein-LAG to immunoglobulins.

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1 Works for me dx.doi.org/10.17504/protocols.io.bjxxkppn

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ABSTRACT

Protein LAG (SpLAG) is an immunoglobulin-binding protein that interacts with the Fc and Fab regions of many mammalian immunoglobulins. It is produced by a chemical coupling of individual proteins and them mixing it up to the appropriate protein ratio. SpLAG binds well to some avian immunoglobulin [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

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MATERIALS

NAME	CATALOG #	VENDOR
Horseradish peroxidase (HRP)	P-100	Gold Biotechnology
Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, V 96 well plate, Non-Treated, clear, without lid, Sterile	260210	Thermo Fisher
Staphylococcal Protein-A		Sigma Aldrich
Protein-L from P. Magnus		

NAME	CATALOG #	VENDOR
Streptococcal protein G by Sigma Aldrich		

1 This ELISA is used to study the interaction of protein-LAG (SpLAG) 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-LAG 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 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.

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