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Universal sandwich enzyme linked immunosorbent assay for investigating protein-AG (SpAG) interactions with immunoglobulins using a SpL-HRP conjugate.

Angel A Justiz-Vaillant[1], Norma McFarlane-Anderson[2]

[1]University of the West Indies St. Augustine; [2]University of West Indies. Mona Campus

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

University of the West Indies angel.vaillant@sta.uwi.edu

Angel Justiz-Vaillant
University of the West Indies St. Augustine

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1 This ELISA is used to study the interaction of a protein-AG (SpAG) with different immunoglobulin preparations from mammalian species.

2 The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of a mixture of protein-A and protein-G in carbonate-bicarbonate buffer pH 9.6.

3 Then plate is treated with bovine serum albumin solution and washed 4X with PBS-Tween.

4 50 µl of animal serum (1 mg/ml) is added and incubated for 1h at room temperature and the microplate is rewashed 4X with PBS-Tween.

5 Then 50 µl of peroxidase-labeled SpL conjugate diluted 1:3000 in PBS-non-fat milk is added to each well and incubated for 1h at RT. The plate is washed 4X with PBS-Tween.

6 50 µl of 4 mg/ml o-phenylenediamine solution (OPD) is added and the plate is incubated 15 minutes at RT in the dark.

7 The reaction is stopped with 50 µl of 3M H_2SO_4 solution.

8 The plate is visually assessed for the development of colour and read in a microplate reader at 492 nm.

9 A cut-off point should be calculated as the mean of the optical density of negative controls x 3. The higher the OD value the higher will be the affinity of SpAG to mammalian immunoglobulins.

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