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Enzyme linked immunosorbent assay for investigating the binding of Protein-L to diverse immunoglobulins

Angel A Justiz-Vaillant[1]

[1]University of the West Indies St. Augustine

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

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

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

ABSTRACT

This SpL ELISA can be used to detect specific antibodies in various animal species including human, mouse, rat, dog, rabbit, chicken, monkey, pig and hamster [1].

1. De Chateau M, Nilson BH, Erntell M, Myhre E, Magnusson CG, Akerstrom B et al. On the interaction between protein L and immunoglobulins of various mammalian species. Scand J Immunol 1993; 37: 399–405

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1 This ELISA is used to study the interaction of protein L with different immunoglobulin preparations.

2 The 96 well microtitre plate is coated overnight at 4°C with 1 µl/mg per well of unlabelled protein-L (SpL) from P. magnus in carbonate-bicarbonate buffer pH 9.6.

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

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4 50 µl of animal serum (1 mg/ml) is added and incubated for 1h at room temperature and washed.

5 Then 50 µl of peroxidase-labeled SpL conjugate diluted 1:5000 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 H2SO4 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.

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