Aug 13, 2020

Universal sandwich enzyme linked immunosorbent assay for investigating immunoglobulin-binding protein (IBP) interactions using a conjugate SpAG-HRP.

DOI
dx.doi.org/10.17504/protocols.io.bjptkmnn

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

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

University of the West In... angel.vaillant@sta.uwi.e...

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

DOI: dx.doi.org/10.17504/protocols.io.bjptkmnn

Protocol Citation: Angel A Justiz-Vaillant, Norma McFarlane-Anderson 2020. Universal sandwich enzyme linked immunosorbent assay for investigating immunoglobulin-binding protein (IBP) interactions using a conjugate SpAG-HRP.. **protocols.io https://dx.doi.org/10.17504/protocols.io.bjptkmnn**

Protocol status: Working
We use this protocol and it's working

Created: August 13, 2020

Last Modified: August 13, 2020

Protocol Integer ID: 40403

1 This ELISA is used to study the interaction of protein-L with different immunoglobulin preparations from avian and mammalian species.

2 The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of SpL 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 SpAG 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 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 SpL to mammalian immunoglobulins.