



Aug 13, 2020

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

DOI

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

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

Protocol Citation: Angel A Justiz-Vaillant, Norma McFarlane-Anderson 2020. Universal sandwich enzyme linked immunosorbent assay for investigating protein-AG (SpAG) interactions with immunoglobulins using a SpL-HRP conjugate..

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Protocol status: Working

We use this protocol and it's working

Created: August 13, 2020

Last Modified: August 13, 2020

Protocol Integer ID: 40407

- 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 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. The higher the OD value the higher will be the affinity of SpAG to mammalian immunoglobulins.