Protein-A and Protein-LG Sandwich ELISA

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1 Works for me dx.doi.org/10.17504/protocols.io.bsu9nez6

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ABSTRACT

This ELISA was used to study the interactions between Staphylococcal protein-A (SpA) and protein-LG (SpLG) with different immunoglobulin preparations from mammalian and avian species.

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1 This ELISA was used to study the interactions between Staphylococcal protein-A (SpA) and protein-LG (SpLG) with different immunoglobulin preparations from mammalian and avian species. The 96 well microtiter plate was coated overnight at 4°C with 2 µg/µl per well of SpA in carbonate-bicarbonate buffer pH 9.6.

2 The plate was then treated with bovine serum albumin solution and washed 4X with PBS-Tween. 50 µl of immunoglobulins (1 mg/ml) is added and incubated for 1h at room temperature, and the microplate is rewashed 4X with PBS-Tween.

3 Then, 50 µL of peroxidase-labeled SpLG conjugate diluted 1:5000 in PBS-non-fat milk was added to each well and incubated for 1h at RT. The plate was washed 4X with PBS-Tween. Then, 50 µL of o-phenylenediamine solution (4 mg/mL) was added, and the plate was incubated for 15 min at RT in the dark.

4 The reaction was stopped with 50 µL of a 3M H2SO4 solution. The plate was visually assessed for color development and read on a microplate reader at 492 nm. A cut-off point was calculated as the mean of the optical density of the negative controls multiplied by two. The cut-off point was set to 0.32.

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