Protein A- Protein A Sandwich ELISA

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1 Works for me
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ABSTRACT

This ELISA was used to study Staphylococcal protein-A (SpA) interactions with various mammalian and avian immunoglobulins.

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1 This ELISA was used to study Staphylococcal protein-A (SpA) interactions with various mammalian and avian immunoglobulins. 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. Fifty microliters of immunoglobulins (1 mg/mL) was added and incubated for 1.30h at room temperature, and the microplate was then rewashed 4X with PBS-Tween.

3 Then, 50 µL of peroxidase-labeled-SpA conjugate diluted 1:5000 in PBS-non-fat milk was added to each well and incubated for 1.30h at RT. The plate was washed 4X with PBS-Tween.

4 Fifty microliters of 3,3',5,5' - tetramethylbenzidine (TMB; Sigma-Aldrich) was pipetted into each well. The reaction was stopped with 50 µL of a 3M H2S O4 solution.

5 The plate was visually assessed for color development and read on a microplate reader at 450 nm. A cut-off point was calculated as the mean of the optical density of the negative controls multiplied by two. The higher the OD value, the higher the affinity of SpA for avian immunoglobulins. The cut-off point was set to 0.30.

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