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# Chimeric Protein-LAG and Protein LA sandwich ELISA

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## ABSTRACT

This ELISA was used to study the interactions between protein-LAG (PLAG) and protein-LA (SpLA) with different immunoglobulin preparations from mammalian and avian species.

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- 1 This ELISA was used to study the interactions between protein-LAG (PLAG) and protein-LA (SpLA) 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 PLAG 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 SpLA 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

- 4 dark. The reaction was stopped with 50 µL of a 3M H<sub>2</sub>SO<sub>4</sub> solution.
- 5 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 value was set to 0.32.