Universal sandwich enzyme linked immunosorbent assay for investigating protein-LG (SpLG) interactions with immunoglobulins using a SpA-HRP conjugate.

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This ELISA is used to study the interaction of a protein-LG (SpLG) with different immunoglobulin preparations from mammalian species.

The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of a mixture of protein-L and protein-G in carbonate-bicarbonate buffer pH 9.6.

Then plate is treated with bovine serum albumin solution and washed 4X with PBS-Tween.

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.

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Then 50 µl of peroxidase-labeled SpA 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.

50 µl of 4 mg/ml o-phenylenediamine solution (OPD) is added and the plate is incubated 15 minutes at RT in the dark.

The reaction is stopped with 50 µl of 3M H2SO4 solution.

The plate is visually assessed for the development of colour and read in a microplate reader at 492 nm.

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 SpLG to mammalian immunoglobulins.