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# Universal sandwich enzyme linked immunosorbent assay for investigating immunoglobulin-binding protein (IBP) interactions using a conjugate SpAG-HRP.

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- 1 This ELISA is used to study the interaction of protein-L with different immunoglobulin preparations from avian and mammalian species.
- 2 The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of SpL 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 SpAG conjugate diluted 1:5000 in PBS-non-fat milk is added to each well and incubated for 1 h 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 H<sub>2</sub>SO<sub>4</sub> 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 SpL to mammalian immunoglobulins.