Sandwich ELISA for investigating the binding of Protein-LG (SpLG) to avian immunoglobulins using anti-IgY-peroxidase as conjugate.

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Protocol status: Working
We use this protocol and it’s working
This ELISA is used to study the interaction of protein-LG (SpLG) with diverse avian immunoglobulins.

The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of recombinant SpLG or a mixture of SpL with SpG 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 avian egg yolk or egg white (1 mg/ml) is added and incubated for 1.30h at RT and the microplate is then rewashed 4X with PBS-Tween.

Then 50 µl of peroxidase-labeled-anti-IgY conjugate diluted 1:15000 in PBS-non-fat milk is added to each well and incubated for 1.30h at RT. After that the plate is washed 4X with PBS-Tween.

Pipette 50 µl of 3,3',5,5' - tetramethylbenzidine (TMB; Sigma-Aldrich) to each well.

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

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A cut-off point can be calculated as the mean of the optical density of negative controls x 3. The higher the OD value the higher will be the binding affinity of SpLG to avian immunoglobulins.