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

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Protocol status: Working
We use this protocol and it's working

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Materials

MATERIALS

- Anti-Chicken IgY, HRP Conjugate, 300ul **Promega Catalog #**G1351
- Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, V 96 well plate, Non-Treated, clear, without lid, Sterile **Thermo Fisher Catalog #**260210
- Staphylococcal Protein-A **Merck MilliporeSigma (Sigma-Aldrich)**
- Protein-L from P. Magnus
- Streptococcal protein G by Sigma Aldrich

Troubleshooting

1 This ELISA is used to study the interaction of protein-LAG (SpLAG) with diverse avian immunoglobulins.

2 The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of a mixture of SpL, SpA and SpG 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 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.

5 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.

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

7 The reaction is stopped with 50 µl of 3M H_2SO_4 solution.

8 The plate is visually assessed for the development of colour and read in a microplate reader at 450 nm.

9 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 SpLAG to avian immunoglobulins.