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Universal sandwich ELISA for investigating the binding of Protein-AG (SpAG) to avian immunoglobulins using a peroxidase-labeled -anti-IgY 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 **Sigma Aldrich**
- Streptococcal protein G by Sigma Aldrich

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

2 The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of recombinant SpAG or a mixture of SpA with 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 room temperature 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 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 binding affinity of SpAG to avian immunoglobulins.