



Aug 14, 2020

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

DOI

[dx.doi.org/10.17504/protocols.io.bjq5kmy6](https://dx.doi.org/10.17504/protocols.io.bjq5kmy6)

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**Protocol Citation:** Angel A Justiz-Vaillant 2020. Universal Sandwich ELISA for investigating the binding of Protein-LAG (SpLAG) to avian immunoglobulins using anti-IgY-peroxidase as conjugate.. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.bjq5kmy6>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** August 14, 2020

**Last Modified:** August 14, 2020

**Protocol Integer ID:** 40445



## 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**

⊗ Protein-L from P. Magnus

⊗ Streptococcal protein G by Sigma Aldrich

- 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<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 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.