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🌐 **Universal sandwich ELISA for investigating the binding of avian and mammalian immunoglobulins to Streptococcal protein-G (SpG) using a peroxidase-labeled Protein LAG conjugate (SpLAG-HRP).**

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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**

⊗ Streptococcal protein G by Sigma Aldrich

Troubleshooting

- 1 This ELISA is used to study the interaction of streptococcal protein-G (SpG) with different avian and mammalian immunoglobulins.
- 2 The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of 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, egg white (1:8 dilutions) or 50 µl of sera or 50 µl of mammalian IgG (1mg/ml) is added to the well 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-Protein-LAG (SpLAG-HRP) conjugate diluted 1:15000 in PBS-non-fat milk is added to each well and incubated for 1.30h at RT. The plate is washed 4X with PBS-Tween.
- 6 Pipette 50 µl of TMB (Sigma-Aldrich) to each well.
- 7 The reaction is stopped with 50 µl of 3M H₂SO₄ 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 affinity of SpG to immunoglobulins.