ABSTRACT

Protein LAG (SpLAG) is an immunoglobulin-binding protein that interacts with the Fc and Fab regions of many mammalian immunoglobulins. It is produced by a chemical coupling of individual proteins and then mixing it up to the appropriate protein ratio. SpLAG binds well to some avian immunoglobulin [1].

References


MATERIALS

- Horseradish peroxidase (HRP) Gold
  Biotechnology Catalog #P-100

- 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 Contributed by
  users

- Streptococcal protein G by Sigma Aldrich Contributed by
  users

Protocol status: Working

We use this protocol and it's working

Created: Aug 19, 2020
This ELISA is used to study the interaction of protein-LAG (SpLAG) with diverse immunoglobulins.

The 96 well microtitre plate is coated overnight at 4°C with 1 µg/µl per well of purified immunoglobulins or 50 µl of any animal sera in carbonate-bicarbonate buffer pH 9.6.

Then plate is treated with bovine serum albumin solution and washed 4X with PBS-Tween.

Then 50 µl of peroxidase-labeled-protein-LAG conjugate diluted 1:5000 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 450 nm.

A cut-off point should be calculated as the mean of the optical density of negative controls x 2 SD.