Mar 13, 2021

Chimeric Protein-LA and Protein LAG sandwich ELISA

Angel A Justiz-Vaillant[1]
[1]University of the West Indies St. Augustine

1 Works for me dx.doi.org/10.17504/protocols.io.bta7nihn

Carbon

Angel Justiz-Vaillant
University of the West Indies St. Augustine

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ABSTRACT

This ELISA was used to study the interactions between protein LA (SpLA) and protein-LAG (PLAG) with different immunoglobulin preparations from mammalian and avian species.

DOI

dx.doi.org/10.17504/protocols.io.bta7nihn

PROTOCOL CITATION

Angel A Justiz-Vaillant 2021. Chimeric Protein-LA and Protein LAG sandwich ELISA . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bta7nihn

CREATED

Mar 13, 2021

LAST MODIFIED

Mar 13, 2021

PROTOCOL INTEGER ID

48191

1 This ELISA was used to study the interactions between protein LA (SpLA) and protein-LAG (PLAG) with different immunoglobulin preparations from mammalian and avian species. The 96 well microtiter plate was coated overnight at 4°C with 2 µg/µl per well of SpLA in carbonate-bicarbonate buffer pH 9.6.

2 The plate was then treated with bovine serum albumin solution and washed 4X with PBS-Tween. 50 µl of immunoglobulins (1 mg/ml) is added and incubated for 1h at room temperature, and the microplate is rewashed 4X with PBS-Tween.

3 Then, 50 µL of peroxidase-labeled PLAG conjugate diluted 1:5000 in PBS-non-fat milk was added to each well and incubated for 1h at RT. The plate was washed 4X with PBS-Tween.

4 Then, 50 µL of o-phenylenediamine solution (4 mg/mL) was added, and the plate was incubated for 15 min at RT in the dark. The reaction was stopped with 50 µL of a 3M H2SO4 solution.

5 The plate was visually assessed for color development and read on a microplate reader at 492 nm. A cut-off point was calculated as the mean of the optical density of the negative controls multiplied by two. The cut-off value was set to 0.30.

Citation: Angel A Justiz-Vaillant (03/13/2021). Chimeric Protein-LA and Protein LAG sandwich ELISAÃÂ . https://dx.doi.org/10.17504/protocols.io.bta7nihn