MAR 13, 2021

Staphylococcal Protein-A and Chimeric Protein-LAG sandwich ELISA

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OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bta5nig6

Protocol Citation: Angel A Justiz-Vaillant 2021. Staphylococcal Protein-A and Chimeric Protein-LAG sandwich ELISA. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bta5nig6>

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Protocol status: Working
 We use this protocol and it's working

Created: Mar 13, 2021

Last Modified: Mar 13, 2021

PROTOCOL integer ID:
 48189

ABSTRACT

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

- 1 This ELISA was used to study the interactions between Staphylococcal protein-A (SpA) 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 SpA 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 μ l 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 μ L 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 μ L 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 μ L 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 0.32.