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Competitive ELISA to study the inhibition of the SpA- binding to Ab-3 by Ab-2.

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Protocol status: Working

We use this protocol and it's working

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Abstract

This immunoassay tested the ability of anti-anti-SpA (Ab-2) to inhibit or interfere with the binding of anti-anti-anti-SpA (Ab-3) to the original antigen- staphylococcal protein A (SpA). It is a confirmation test of the functional capacity of Ab-2. In this ELISA, both Ab-2 and the antigen SpA compete for binding to Ab-3. When Ab-2 is present, it blocks the binding of HRP-labeled SpA to Ab-3, resulting in inhibition of reaction color development.

Guidelines

Use appropriate positive and negative controls, as well as blanks.

Materials

MATERIALS

⊗ Substrate (TMB) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #EZMADP-60K(kit)**

⊗ Stop Solution (0.3M HCl) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #EZMADP-60K(kit)**

⊗ 96-Well Microtiter[®]; Microplates, Polystyrene, 280µL, Nonsterile, V-bottom **Thermo Fisher Catalog #2605**

⊗ Pierce[®]; Recombinant Protein A, Peroxidase Conjugated **Thermo Fisher Catalog #32400**

Troubleshooting

Safety warnings

❗ Do not use any reagent where damages to the packaging has occurred.



- 1 Prepare materials and ELISA buffer solutions and reagents.
- 2 Pipette 53 μ l of purified Ab-3 (100 μ g/ml) mixed with 2.8 ml of coating buffer into each well.
- 3 Incubate the microplate at 37°C for 4 hrs.
- 4 Aspirate the contents of the wells.
- 5 Fill each well with an appropriately diluted washing solution and aspirate.
- 6 Wash the microplate 3 times.
- 7 Pipette 53 μ l of serial dilutions of pooled Ab-2 (100 μ g/ml) in triplicates.
- 8 Incubate the microplate at RT for 1 hr.
- 9 Rewash the microplate filling each well with 100 μ l of washing buffer.
- 10 Pipette 53 μ l of commercially available peroxidase-labeled SpA conjugate (Sigma-Aldrich) diluted 1:5000 to each well.
- 11 Reincubate the microplate at RT for 1 hr.
- 12 Repeat step 5.
- 13 Pipette 53 μ l of TMB (Sigma-Aldrich) to each well.



- 14 Incubate in the dark at RT for 14 min.
- 15 Measure absorbance at 450 nm in a microplate reader and analyze the results