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

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

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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.

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GUIDELINES

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

MATERIALS

NAME	CATALOG #	VENDOR
Substrate (TMB)	EZMADP-60K(kit)	Millipore Sigma
Stop Solution (0.3M HCl)	EZMADP-60K(kit)	Millipore Sigma
96-Well Microtiter™ Microplates, Polystyrene, 280µL, Nonsterile, V-bottom	2605	Thermo Fisher
Pierce™ Recombinant Protein A, Peroxidase Conjugated	32400	Thermo Fisher

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 µl of purified Ab-3 (100 µg/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 µl of serial dilutions of pooled Ab-2 (100 µg/ml) in triplicates.

8 Incubate the microplate at RT for 1 hr.

9 Rewash the microplate filling each well with 100 µl of washing buffer.

10 Pipette 53 µl 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 µl of TMB (Sigma-Aldrich) to each well.

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14 Incubate in the dark at RT for 14 min.

15 Measure absorbance at 450 nm in a microplate reader and analyze the results

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