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ELISA for anti-SpA antibodies in hyper-immune egg whites.

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1 Works for me dx.doi.org/10.17504/protocols.io.bjnrkmd6

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MATERIALS

NAME	CATALOG #	VENDOR
96-Well Microtiter™ Microplates, Polystyrene, 330µL, Nonsterile, Flat-bottom	9205	Thermo Fisher
3 3'5 5'-Tetramethylbenzidine Liquid Substrate Supersensitive for ELISA	T4444-100ML	Sigma Aldrich
Staphylococcal Protein-A		Sigma Aldrich
Anti-Protein-A		

1 The 96 well polystyrene microplate (U-shaped bottom) is coated with 500 ng of SpA (Sigma-Aldrich) in coating buffer for 4 h at 37oC.

2 The microplate is washed four times with PBS-Tween-20 and blocked with 3% non-fat milk in PBS, 25 µl/well, 1h, RT.

3 The microplate is washed four times again.

4 Samples of egg whites are added in 1:10 dilutions.

5 After incubation for 1h at RT the microplate is washed four times.

6 Then, 50 µl of peroxidase-labelled protein-A diluted 1:3000 (Sigma-Aldrich) is added.

7 The microplate is incubated for 1h at RT.

8 The microplate is washed four times.

9 Tetramethylbenzidine (TMB) solution (50 µl) is added to each well.

10 Perform a further incubation of 15 min in the dark

11 The reaction is stopped with 3M H_2SO_4.

12 Read the microplate in a microplate reader at 450 nm.

13 Calculate the cut-off point as appropriate.

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