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

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Protocol status: Working

We use this protocol and it's working

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Materials

MATERIALS



96-Well Microtiter[®]; Microplates, Polystyrene, 330µL, Nonsterile, Flat-bottom **Thermo Fisher Catalog #9205**



3,3',5,5'-Tetramethylbenzidine Liquid Substrate Supersensitive for ELISA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T4444-100ML**



Staphylococcal Protein-A **Merck MilliporeSigma (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 37°C.
- 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 H₂SO₄.
- 12 Read the microplate in a microplate reader at 450 nm.
- 13 Calculate the cut-off point as appropriate.