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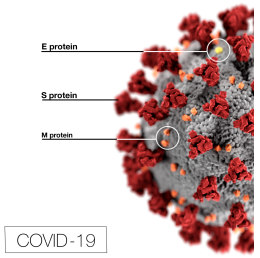
Enzyme-linked immunosorbent assay using the SARS-CoV-2 membrane glycoprotein analog M1s (sequence: ac-CTITVEELKKLLEQC-am) for detection of cognate COVID-19 antibodies as performed on high binding plates



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Coronavirus Method De...

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We use this protocol and it's working

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Abstract

This protocol describes a peptide-based indirect enzyme-linked immunosorbent assay (ELISA) for detecting antibodies against the SARS-CoV-2 membrane glycoprotein analog M1s (synthetic peptide sequence: ac-CTITVEELKKLLEQC-am). High-binding polystyrene plates are coated overnight with the peptide antigen in carbonate-bicarbonate buffer, followed by blocking, incubation with diluted serum, detection using Protein A–peroxidase, and colorimetric readout with TMB substrate at 450 nm. The method offers a simple, reproducible, and cost-effective workflow for COVID-19 serological testing using synthetic peptide antigens. Expected results include distinct absorbance profiles proportional to antibody levels, supporting applications in seroprevalence analysis, immune response monitoring, and assay validation for SARS-CoV-2 antibody research.

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Coat

1 overnight incubation at 4°C
100µL/well, Carbonate-bicarbonate buffer in water, pH 9.6

Wash

2 3x at 5 min interval
0.05% Tween 20 in 1X PBS (PBST)
freeze

Block

3 *leave plates at RT for 15min before blocking*
incubation at 37°C for 30 mins
200µL/well, 5% skimmed milk in PBST

Wash

4 3x at 5 min interval
0.05% Tween 20 in 1X PBS

Primary antibody

5 incubation at RT for 1hr
100µL/well, 1:100 sera in dilution buffer (0.05% skimmed milk in PBST)

Wash

6 3x at 5 min interval
0.05% Tween 20 in 1X PBS

Secondary antibody

7 incubation at RT for 1hr
50µL/well, 1:2000 Protein A-peroxidase (0.5ug/mL) in dilution buffer

Wash

8 3x at 5 min interval
0.05% Tween 20 in 1X PBS

Chromogenic substrate solution

9 incubation at RT for 30 min
50µL/well, 0.1mg/mL TMB in Phosphate Citrate Buffer in water, pH 6.0

Stop solution

10 50µL/well, 1M H_2SO_4
read at 450nm