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Synthetic peptide-based indirect ELISA using S1c (of the sequence CPVAIHADQLTPTWRVYSTC) as the antigen, performed on regular polystyrene plates

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We use this protocol and it's working

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Disclaimer

Abstract

This protocol describes a synthetic peptide-based indirect **enzyme-linked immunosorbent assay** (ELISA) using the peptide **S1c (sequence: CPVAIHADQLTPTWRVYSTC)** as the immobilized antigen. The assay is designed for antibody detection in serum samples and uses regular polystyrene microplates. Plates are coated overnight at 4 °C with 100 µL/well of S1c in carbonate–bicarbonate buffer (pH 9.6), then washed thrice with 0.05% Tween 20 in PBS (PBST). After a 15 min equilibration to room temperature, wells are blocked with 200 µL/well of 5% skimmed milk in PBST for 1 h at 37 °C and washed again three times. Primary antibody incubation is performed for 1 h at room temperature using 100 µL/well of sera diluted 1:100 in 0.05% skimmed milk–PBST, followed by washing. Detection is achieved with Protein A–peroxidase (0.5 µg/mL) diluted 1:2000 in PBST, incubated for 1 h at room temperature. After a final wash, the reaction is developed with 0.1 mg/mL TMB in phosphate–citrate buffer (pH 6.0) for 1 h at room temperature, stopped with 50 µL of 1 M H_2SO_4, and absorbance is measured at 450 nm. This optimized workflow enables reliable detection of anti-S1c antibodies using accessible reagents and standard ELISA plates.

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 1hr
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

Substrate

9 incubation at RT for 1hr
50µL/well, 0.1mg/mL TMB in Phosphate Citrate Buffer in water, pH 6.0

Stop

10 50µL/well, 1M H2SO4
read at 450nm