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SARS-CoV-2 RBD ELISA

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MATERIALS TEXT

Reagents

Plates

1. Falcon® 96-well Clear Round Bottom TC-treated Cell Culture Microplate, with Lid, Individually Wrapped, Sterile, 50/Case (Corning, Falcon product number: 353077)
2.Clear Flat-Bottom Immuno Nonsterile 384-Well Plates (Thermofisher, 464718)

Antigens

SARS-CoV-2 RBD
Stock concentration: 1mg/mL

Antibodies

CR3022- IgG, IgA and Igm mAb
Goat anti-human IgG-HRP (Jackson Immunoresearch109-035-098)
Goat anti-human IgA-HRP (Jackson Immunoresearch 109-035-011)
Goat anti-human IgM-HRP (Jackson Immunoresearch 109-035-129)

Buffers: (see recipes at end of protocol)

Coating buffer: Carbonate buffer pH to 9.6
Wash Buffer: 50 mM Tris, 400 mM NaCl, 0.05% Tween pH 8 (1X high salt TBST)
Blocking buffer: 5% Milk 1X TBS pH 8 (make fresh, needs at least 30 minutes to go in solution)
Sample buffer: 5% Milk in 1X TBS -0.05% Tween pH 8 (make fresh, needs at least 30 minutes solubilize)
Substrate buffer: 1-Step™ Ultra TMB-ELISA Substrate Solution(Thermofisher, catalog: 34029)
STOP solution: 2N stop H_2SO_4

Miscellaneous

Plate seals
Reservoirs
Multichannel pipettes (30-300ul, 10-100ul capacity).

Regular pipettes of all sizes (P1000, P200, p20, P2)
Sterile tips all capacity
Aluminum foil.

Coating buffer:Carbonate buffer (for 1 Liter)
1.59g Na2CO3, 2.93g NaHCO3. Bring to 900ml with water. Adjust pH to 9.6 and bring the final volume to 1 liter. Re-check pH. Sterile filter (0.2 um) or autoclave.

10X TBS (500 mM Tris, 1.4 M NaCl) for 1L
60.57g Tris base
181.14g NaCl
pH to 8.0

1X TBS (for 1L)
100mL 10X TBS
900mL ddH20

10X high salt TBS (500 mM tris, 4M NaCl) for 1L
60.57g Tris base
233.76 g NaCL
pH to 8.0

1X High salt TBS-0.05% Tween (for 1L)
100mL 10X high salt TBS
900mL ddH20
500ul Tween

5% Milk-1X TBS- 0.05% Tween (For 50mL)
2.5g Non-fat powdered Milk
25 uL Tween
Bring volume to 50mL with 1X TBS (sterile)
Do not vortex, mix by stirring or inversion
Do not autoclave or sterile filter

5% Milk-1X TBS (for 50 mL)
2.5g Non-fat powdered Milk
Bring volume to 50mL with 1X TBS (sterile)
Do not vortex, mix by stirring or inversion
Do not autoclave or sterile filter

1 **Before you start:**
-Refer to the materials and recipes prior to starting the experiment.
-Critical that carbonate buffer, sample diluent, substrate, and substrate buffer be at room temperature before use.
-Make blocking and sample buffer 30 mins before assay to allow solubilization of milk powder.

2 **Coating:**
-Dilute SARS-CoV-2 RBD antigen to 1 µg/mL in carbonate buffer (20 mL per 384 well plate).
-Add 50 µl/well of this antigen to a 384 well plate (200µl/ well for 96 well plate).
-Cover with plate seal. Using a microcentrifuge, quick spin plates to allow the solution to settle at the bottom.
-Incubate at room temperature for 1 hour.
-Wash 3x (300 µl/well for 96 well plate, 100µl/well for 384 well plate) in high salt TBST and blot plate dry on paper towels.

3 **Blocking:**
-Add 5% Milk-1X TBS (96 well plate: 300 µl/well; 384 well plate: 100 µl/well) to the assay plate.
-Cover with plate seal. Using a microcentrifuge, quick spin plates to allow the solution to settle at the bottom.
-Incubate at room temperature for 30 minutes.

-During the blocking incubation, prepare samples and standards by diluting in 5% Milk- 1X TBST as appropriate or indicated below.
-At the end of 30 mins, wash the plate 3x in high salt TBST (300 µl/well for 96 well plate, 100µl/well for 384 well plate) and blot plate dry on paper towels.

4 **Samples and Standard Preparation and Addition:**

-Serially dilute samples 1:100, 1:400, 1:1600 and 1:6400 in 5% Milk- 1X TBST.
-For monoclonal standards, perform a 2-fold, 8 series dilution starting at 0.025 µg/ml for IgG and 0.25 µg/ml for IgA and IgM. Transfer standards to 96 well round-bottom plate.
-At the end of blocking step, transfer samples and standards (96 well plate: 100 ul/well; 384 well plate: 25 ul/well) to the assay plate that was coated with RBD and blocked in steps above.
-Cover with plate seal. Using a microcentrifuge, quick spin plates to allow the solution to settle at the bottom.
-Incubate for 1 hour at 37°C on a plate shaker set to 100 rpm.
At the end of 1-hour incubation, wash 5x in high salt TBST (300 µl/well for 96 well plate, 100µl/well for 384 well plate) and blot plate dry on paper towels.

5 **Secondary:**

-Add secondary at the following dilutions in 5% Milk-1X TBST (96 well plate: 100 ul/well; 384 well plate: 25 ul/well)
a.IgG 1:10,000
b.IgA 1:5,000
c.IgM 1:10,000
-Cover with plate seal. Using a microcentrifuge, quick spin plates to allow the solution to settle at the bottom.
-Incubate for 30 minutes at room temp on a plate shaker set to 100 rpm.
-Wash 5x in high salt TBST and once with 1X TBS (300 µl/well for 96 well plate, 100µl/well for 384 well plate) and blot plate dry on paper towels.

6 **Development**

-Make sure TMB equilibrated to room temp 30 mins before development.
-Add appropriate volume of TMB to wells (96 well plate: 100 ul/well; 384 well plate: 25 ul/well). Start a timer as soon as you add substrate to the first set of wells.
-Cover with plate seal and spin down using a microcentrifuge. Incubate in dark for 5 minutes.
-Add 2N stop H_2SO_4stop solution (96 well plate: 100 ul/well; 384 well plate: 25 ul/well)
-Read plates at 450 nM – 570 nm.