SARS-CoV-2 ELISA Key Reagent Kit

Catalog number: D3201

Available in 3 sizes: sufficient for **5 microplates**, **20 microplates**, or **50 microplates**

The kit detects and quantitates human IgG antibodies that react with SARS-CoV-2 spike protein. Human IgM or IgA, or non-immunoglobulin plasma are not detected.

***** This assay is specific for human IgG Fc, and does not cross react with mouse, rat, rabbit, cat and dog Fc.

* This package insert must be read in its entirety before using this product.

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MANUFACTURED AND DISTRIBUTED BY:

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). When humans are exposed to the virus, they can acquire immunity by forming IgG antibodies against the spike protein on the viral envelope. It is vital to know which individuals are already exposed to the virus by testing for the human IgG's that are present in the serum against the viral spike protein. This reagent kit is developed to detect the human IgG's against SARS-CoV-2 in plasma and serum, and help develop other diagnostic and research tools to study immunity against SARS-CoV-2. The kit only contains the key reagents necessary (spike protein, secondary antibody, and standard ACE2-Fc protein and positive control MD-10 that are known to bind to the spike protein), and these reagents can be used with any ELISA-compatible plates and equipment, and other generic buffers and reagents for HRP detection.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. The capturing SARS-CoV-2 protein is coated onto a 96-well microplate. Standards, control, and samples are pipetted into the wells and any spike-binding protein is bound to the immobilized spike protein. After washing away any unbound substances, an HRP-linked polyclonal antibody specific for human IgG Fc is added to the wells. Following an incubation and a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution is added. The intensity of the color measured is in proportion to the amount of spike-binding antibody bound in the initial step. The antibody level in the blood sample is reported as the dilution factor necessary to reach a certain percentage of the maximum signal reached with the standard protein ACE2-Fc.

PRECAUTION

The Stop Solution provided in this kit is a solution of low pH. Wear eye, hand, face, and clothing protection when using this material.

MATERIALS PROVIDED

	DESCRIPTION	D3201 5 plates	D3201 20 plates	D3201 50 plates
1	**Capturing Protein** – SARS-CoV-2 spike protein	1 vial (125 µL)	1 vial (500 µL)	2 vials (625 µL/vial)
2	**HuFc Detection Antibody** - a horseradish peroxidase (HRP)-conjugated polyclonal antibody against human IgG Fc protein	1 vial (25 µL)	1 vial (100 µL)	1 vial (250 µL)
3	**MEDNA ACE2-Fc Standard Protein (300 ng/mL)**	1 vial (50 µL)	1 vial (200 µL)	1 vial (500 µL)
4	**MEDNA MD10 Positive Control (300 ng/mL)**	1 vial (50 µL)	1 vial (200 µL)	1 vial (500 µL)

MATERIALS NOT PROVIDED

	DESCRIPTION
1	**96-well or 384-well ELISA Microplate**
2	**50 mM $NaHCO_3$, pH 9.5, Coating Buffer**
3	**3% BSA in 1 x PBS Blocking Buffer**
4	**Wash Buffer** - PBS
5	**Assay Diluent** - PBS with 1% BSA
6	**Color Reagent** - Tetramethylbenzidine (TMB)
7	**Stop Solution** - 0.5 N Hydrochloric acid solution

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, optionally with correction wavelength set at 540 or 570 nm
- Pipettes and pipette tips
- Polypropylene test tubes
- Deionized or distilled water

STORAGE

- Do not freeze
- Store at 2-8 ℃
- Best if used within 3 months of receipt

MICROPLATE PREPARATION PROCEDURE

1. For each 96-well microplate, prepare 5 mL of sodium bicarbonate buffer (50 mM, pH 9.5) containing 2 µg/mL of Capturing protein, and aliquot 50 µL per well. Incubate overnight at 4ºC. Alternatively, use PBS in place of carbonate/bicarbonate buffer for coating.
2. Remove liquid and wash wells once with PBS (200 µL per well).
3. Block plate with 3% BSA in PBS (200 µL per well) for at least 1 hour at room temperature.
4. Remove liquid and wash wells once with PBS (200 µL per well).
5. Coated plates can be stored foil wrapped at 4ºC for up to 2 weeks.

SAMPLE PREPARATION

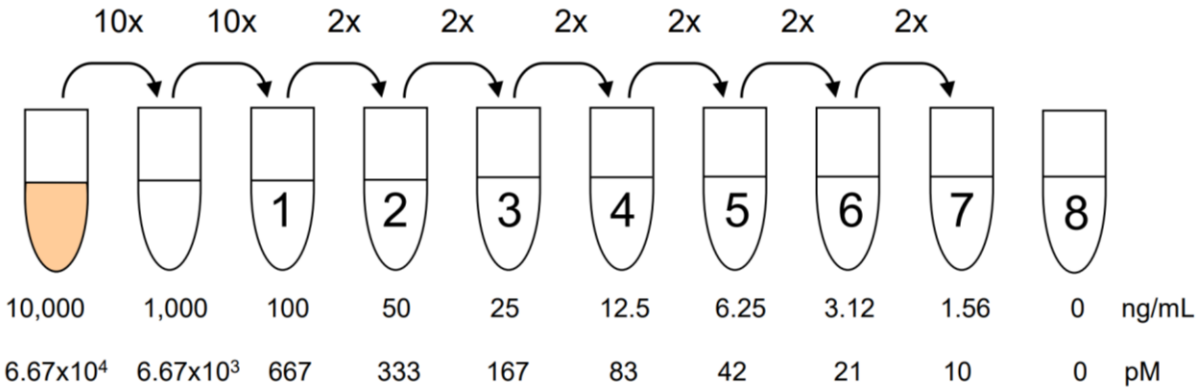
Cell culture supernatants - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20℃. Avoid repeated freeze-thaw cycles.
Serum - Allow samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifuging for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or store samples at ≤-20℃. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20℃. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

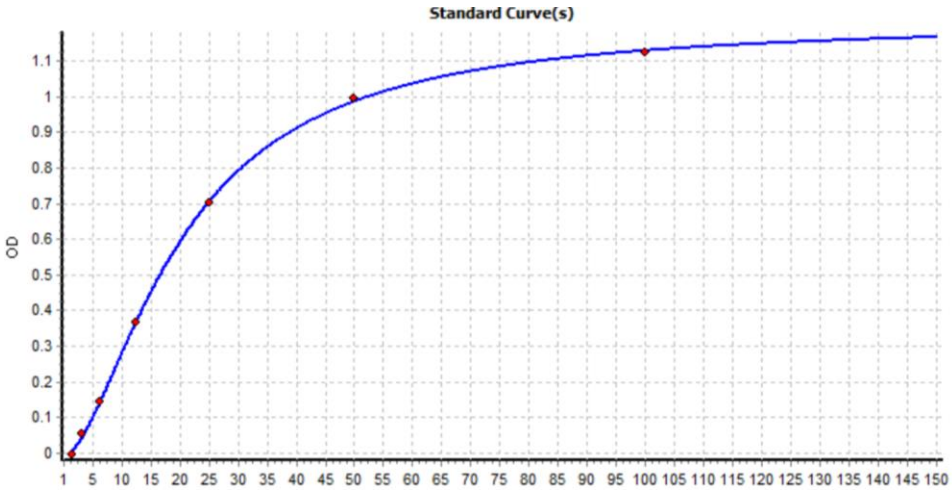
1. Bring all reagents to room temperature before use.
2. Make appropriate dilutions of ACE2-Fc Standard in Assay Diluent. Also make appropriate dilution of MD10 positive control.
3. Make appropriate dilution of test samples in Assay Diluent (1% BSA in PBS).
4. Prepare 1x PBS as Wash Buffer.
5. Prepare Detection Antibody Solution by diluting Detection Antibody 1:3000 in Assay Diluent.

6. Prepare 0.5 N Hydrochloric acid as Stop Solution

ACE2-Fc standard serial dilution:



Sample protein standard curve fit (4-PL fit):



MEDNA protein standard (ng/mL)

Note on standard curve linearity: The linearity of standard curves is influenced by various factors including incubation time, temperature, amount of secondary antibody, and reaction substrate. With advanced data analysis software, standard curves do not need to be perfectly linear for suitable data analysis and conversion. MEDNA uses and recommends BMG LABTECH Microplate Readers and MARS Data Analysis Software (www.bmglabtech.com).

ASSAY PROCEDURE

1) Prepare reagents, standard curve serial dilution, and test samples as directed in the previous section.
2) Cover the unused microplate wells with plastic seal for future use.
3) Add 50 µL/well of standard dilutions, control, and appropriately diluted samples.
4) Incubate for 1 hour or longer at room temperature, or for 30 minutes in 37°C.
5) Remove liquid from wells.
6) Wash each well 3 times with 200 µL/well of **1x Wash Buffer.**
7) Add 50 µL/well of **HuFc Detection Antibody.**
8) Incubate for 30 minutes or longer at room temperature, or in 37°C.
9) Remove liquid from wells.
10) Wash each well 3 times with 200 µL/well of **1x Wash Buffer.**
11) Add 50 µL/well of **Color Reagent.**
12) Incubate at room temperature until color develops (2-15 minutes).
13) Read absorbance on a microplate reader at 650 nm (optional).
14) Add 50 µL/well of **Stop Solution**

    (CAUTION: STOP SOLUTION IS ACIDIC; WEAR PROPER PROTECTION)
15) Read absorbance on a microplate reader at 450 nm immediately.
16) Use 540 nm or 570 nm for wavelength correction (optional).

MATERIAL SAFETY DATA
Hazard information is provided for compliance with both the UK Chemicals (Hazard Information and Packaging) (CHIP) Regulations and the US Hazard Communication Standard (HCS)

Hazards Identification:
STOP SOLUTION (0.1 N hydrochloric acid) is a diluted hydrochloric acid. Hydrochloric acid is a hazardous material. CAS NO.7647-01-0. Molecular Weight: 36.46 Chemical Formula: HCl

Emergency Overview
POISON! DANGER! CORROSIVE. LIQUID AND MIST CAUSE SEVERE BURNS TO ALL BODY TISSUE. MAY BE FATAL IF SWALLOWED OR INHALED. INHALATION MAY CAUSE LUNG DAMAGE.

SAF-T-DATA(tm) Ratings (Provided here for your convenience)

Health Rating: 3 - Severe (Poison)
Flammability Rating: 0 - None
Reactivity Rating: 2 - Moderate
Contact Rating: 4 - Extreme (Corrosive)
Lab Protective Equip: GOGGLES & SHIELD; LAB COAT & APRON; VENT HOOD; PROPER GLOVES
Storage Color Code: White (Corrosive)

Personal Protection
Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, and gloves. Avoid contact of material with skin or eyes. Ensure access to a safety shower and eye-wash.

First Aid Measures
Inhalation: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.
Ingestion: DO NOT INDUCE VOMITING! Give large quantities of water or milk if available. Never give anything by mouth to an unconscious person. Get medical attention immediately.
Skin Contact: In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.
Eye Contact: Immediately flush eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

UNIT CONVERSION

Molarity of Standards:

The ACE2-Fc serial dilution is equivalent to:

$6.67x10^4$, $6.67x10^3$, 667, 333, 167, 83, 42, 21, 10, 0 pM

Conversion of (X) ng/mL to (Y) nM:

$$\frac{X\ ng/mL}{MW\ (g/mol)} = \frac{X\ ng/10^{-3}\ L}{MW\ (ng/nmol)} = Y\ nM$$

Example:

Sample concentration: 120 ng/mL

MW of Sample: 150 kD

$$\frac{120\ ng/mL}{150{,}000\ (g/mol)} = \frac{120\ ng/10^{-3}\ L}{150{,}000\ (ng/nmol)} = 0.8\ nM$$

If molecular weight of sample differs from ACE2-Fc, adjust the sample reading concentration according to the ratio in molecular weight difference. Example:

MW of ACE2-Fc: 150 kD
MW of Sample: 75 kD
Calculated concentration of sample: 40 ng/mL

$$\frac{[75{,}000\ g/mol]}{[150{,}000\ g/mol]}\ X\ 40\ ng/mL = 20\ ng/mL$$

If the molecular weight of the sample is smaller than that of the MEDNA standard, then the actual concentration of the sample is lower than the sample reading concentration.