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User's Manual

SARS-CoV-2 Antigen ELISA Kit

REF **DEIA2020**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit is intended for the quantitative detection of the recombinant SARS-COV-2 nucleoprotein antigen in human serum. It can recognize recombinant SARS-CoV Nucleoprotein / NP Protein, but no react with recombinant MERS-CoV Nucleoprotein / NP protein. The use of this kit for natural samples need be validated by the end user due to the complexity of natural targets and unpredictable interference.

General Description

Coronaviruses are enveloped viruses with a positive-sense RNA genome and with a nucleocapsid of helical symmetry. Coronavirus nucleoproteins localize to the cytoplasm and the nucleolus, a subnuclear structure, in both virus-infected primary cells and in cells transfected with plasmids that express N protein. Coronavirus N protein is required for coronavirus RNA synthesis, and has RNA chaperone activity that may be involved in template switch. Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid. Nucleocapsid protein is a highly immunogenic phosphoprotein also implicated in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N protein of coronavirus is chosen as a diagnostic tool.

Principles of Testing

The new Coronavirus (SARS-COV-2) antigen detection kit is based on the principle of a double antibody sandwich enzymelinked

immunoassay. The microplate is pre-coated with an anti-new coronavirus N protein antibody. When the serum and plasma to be detected contain the new coronavirus N protein antigen, it will bind to the specific antibody coated on the microplate. After the secondary antibody against the new coronavirus N protein was added, an "antibody-antigen-enzymelabeled antibody" complex is formed. After adding the chromogenic substrate TMB, HRP enzyme will catalyze the color development. After terminating the solution, the microplate reader will determine the Absorbance value to determine the presence or absence of new coronavirus antigen in each test sample.

Reagents And Materials Provided

1. The Antibody coated microplate: 12x8 strips
2. Positive control: 1 mL
3. Negative control: 1.5 mL
4. Sample diluent: 12 mL
5. HRP-Enzyme conjugate: 12 mL
6. TMB substrate: 12 mL
7. Stop solution: 6 mL
8. Wash buffer(20x): 50 mL

9. Cover film: 2 pieces
10. Seal bag: 2 pieces
11. Insert: 1 piece

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm
2. Pipettes and pipette tips
3. Deionized or distilled water
4. Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer

Storage

The kit should be stored at 2 ~ 8 °C, protected from light, and valid for 6 months.

Specimen Collection And Preparation

Fresh serum and plasma collected from human veins can be used for samples. The samples can be refrigerated at 2-8 ° C within 48 hours after specimen collection. If not tested immediately, long-term storage is required. It can be stored for six months at below -20 ° C. Do not add preservative sodium azide.

Reagent Preparation

1. 1x wash buffer

Dilute the concentrated wash buffer to 1000 mL with deionized water.

Assay Procedure

1. Equilibrium: Recover samples, microplates and required reagents for 30 minutes at room temperature.
2. Sample incubation: Fix the required microwells on the rack. For each test, 3 wells of negative control and 2 wells of positive

control were set up, and 100 µL of the corresponding reference substance was added. Add 90µL of the sample dilution and 10µL of the serum and plasma samples to the other wells. Cover the plate and incubate at 37 ° C for 60 minutes.

3. Washing: Discard the liquid in each well, fill the microwells (300 µL/well) with 1x wash buffer, and discard the liquid in the

well after standing for 30 seconds; repeat 5 times, and pat dry on the tissue paper after the last washing.

4. Add HRP-enzyme conjugate: Add 100µL of HRP-enzyme conjugate to each well, seal the plate with a cover film and place it in 37°C, incubate for 30 min.
5. Washing: Discard the liquid in each well, fill the microwells (300 µL/well) with 1x wash buffer, and discard the liquid in the

well after standing for 30 seconds; repeat 5 times, and pat dry on the tissue paper after the last washing.

6. Color development: Add 100 µL of TMB substrate to each well, mix by shaking slightly, and set the color at 37°C in the dark

for 30 minutes.

7. Measurement: Add 50 µL of stop solution to each well. Select the microplate reader with the main wavelength of 450nm and the reference wavelength of 630nm to determine the absorbance of each well.

Quality Control

The mean value of the positive control OD is ≥1.0, and the negative control value is ≤0.10, then the test is valid; otherwise, it is deemed invalid, and the test should be repeated.

Interpretation Of Results

Cut-off value = 0.10 + average OD value of negative control

When the average OD value of the negative control is less than 0.05, it is calculated as 0.05. If the average OD value is more than 0.05, should calculate according to the actual value.

Positive Results: Samples OD value ≥ Cut-off value. Samples with absorbance value equal or greater than the Cut-off value are considered initially reactive, which indicates that SARS-COV-2 antigens have probably been detected.

Negative Results: Sample OD value ≤ Cut-off value. Samples with absorbance value less than the Cut-off value are negative for this assay, which indicates that no SARS-COV-2 antigens have pbeen detected.

Typical Standard Curve

Reproducibility

The assay was repeated 10 times with a CV less than 15%.

Three lots were tested with the same samples 10 times with a CV less than 15%.

Precautions

1. The kit should be taken out from the refrigerated environment and should be equilibrated to room temperature before being opened for use. The reagent should be thoroughly shaken before use.
2. The strips of the kit can be removed, and the unused pre-coated strips should be sealed in a desiccant bag.
3. Do not mix reagent components from different batches of the kit.
4. If crystals appear in the 20-fold concentrated washing solution, they should be placed at 37 ° C until the crystals are

completely dissolved before use.

5. Read the results of the microplate reader within 30 minutes after the reaction is terminated.

6. This kit and all waste in the test are potentially contaminating and should be treated strictly in accordance with medical contamination.

Limitations

1. This product is only used for testing of serum or plasma samples.
2. The test results of this product are for clinical reference only, and they should not be used as the sole basis for the diagnosis of new coronavirus.
3. Due to the window effect of the virus infection and the sensitivity of the kit detection, a negative test result cannot exclude the possibility of virus infection.