Creative Diagnostics®

Version 01-06/20

User's Manual

SARS-CoV-2 Neutralizing Antibody Assay Kit

REF **DEIASL055**

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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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The RBD Neutralizing Antibody competitive ELISA Kit is an enzyme-linked immunosorbent assay intended for the qualitative detection of an RBD neutralizing antibodies in serum or plasma.

General Description

S protein contains a receptor-binding domain(RBD) which is one of the vital immunodominant epitopes and has a superior capacity to induce neutralizing antibodies. It is proved that RBD is responsible for recognizing and interacting with he cell surface receptor, angiotensin-converting enzyme-2 (ACE2). Following the binding of the RBD to the receptor ACE, pathogen enters target cells, where the fusion of the virus envelops the endosome membranes and leads to the release of the viral nucleocapsid into the cytosol of the infected cell.

Principles of Testing

The Neutralizing Antibody Competitive ELISA Kit is a competitive ELISA detection tool. The test kit contains microplate strips each with 8 wells coated with human ACE2 receptor protein (HACE2). In the first reaction step, samples are diluted with horseradish peroxidase Hrphabelled recombinant RBD protein (HRP-RBD) and incubated in the supplied 96-well diluting plate, in the case of positive samples, neutralizing antibodies will bind to the HRP-RBD. To detect the unbound HRP-RBD, reaction mixture is transferred into the ELISA microplate to catalyze a color reaction. The interaction between HRP-RBD and HACE2 can be blocked by neutralizing antibodies bound to RBD. The final solution can be read by a microplate reader at wavelength of 450 nm, and the absorbance of the samples is inversely dependent on the concentration of the active neutralizing antibodies, and the inhibition can be calculated according to the OD450 value of samples and negative control.

Reagents And Materials Provided

1. Microplate wells coated with hACE2: 12x8, 96wells, ready-to-use.
2. 96-well diluting plate: 96well
3. HRP conjugated RBD, HRP-RBD (100x concentrate): 100 µl
4. HRP dilution buffer: 10 ml, ready-to-use
5. Sample dilution buffer: 10 ml, ready-to-use
6. Wash buffer (80x concentrate): 30 ml
7. Positive control (Neutralizing antibody, human): 50 µl, ready-to-use
8. Negative control: 50 µl, ready-to-use
9. TMB Substrate solution: 10 ml, ready-to-use
10. Stop Solution: 6 ml, ready-to-use
11. Protective foil: 3 pieces
12. Instruction for use: 1 booklet

Note: The components in different batches of kits are not interchangeable. The format of each reagents is the minimum volume.

Materials Required But Not Supplied

1. Microplate reader: wavelength of 450 nm
2. Automatic microplate washer: recommended, the washing step can also be camied out manually.
3. Microplate shaker
4. Calibrated pipettes
5. Pipette tips
6. Distilled or deionized water
7. Incubator or water bath
8. Timer
9. Paper towel
10. Disinfecting agent

Storage

1. The test kit has to be stored at a temperature between +2°C and +8°C, do not freeze, and avoid exposure to direct sunlight. The unopened Is stable for at least 10 months. The opened kit can be stored at a temperature between +2°C and +8°C for 2 weeks.
2. The opened positive control can be stored at a temperature between+2°C and +8°C for 7 days. For longer storage, please aliquot and freeze at-20°C for no longer than 3 months, avoid repeated freeze-thaw cycles.

Specimen Collection And Preparation

Samples: Serum or plasma.

1. The sediment and suspended matter in samples may interference with the test results, which should be removed by centrifugation at 6000 x g, 10 min.
2. Severe hemolysis, lipoid, or turbidity samples should not be used.
3. Samples should be stored at room temperature no longer than 8 hours, if the assay will not be completed within 8 hours, the samples should be refrigerated at +2°C to +8°C. if the assay will not be completed within 48 hours, or if the samples will be stored for over 48 hours, samples should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed overheated.
4. Frozen samples must be mixed well after thawing and brought to room temperature(+18°C to +28°C)before testing.

Assay Procedure

Assay Preparation

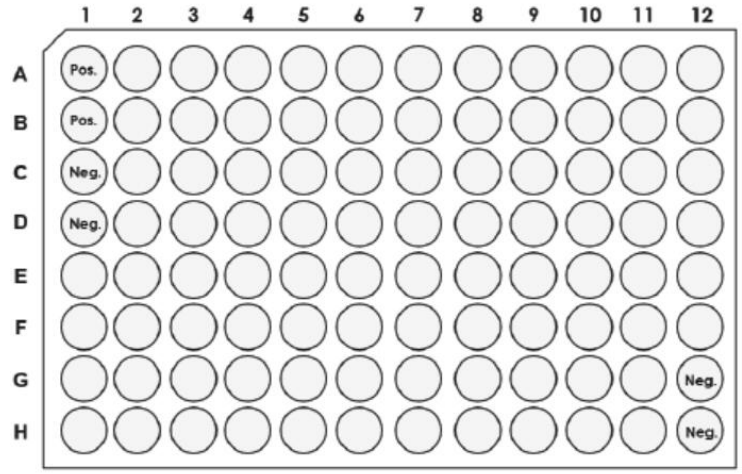
1. All reagents must be brought to room temperature (+18°C to +28°C) at least 30 min before use. Keep all reagents in refrigerator promptly after use.

2. Wash buffer: The wash buffer is 80 x concentrate, please diluted 30 ml concentrate wash buffer with 2370 ml deionized or distilled water (1 part reagent plus 79 parts water) before use.
3. HRP conjugated RBD: The HRP-RBD is a 100 x concentrate, please dilute with a 1: 99 ratio with HRP dilution buffer. Add 100 µl of HRP-RBD in HRP dilution buffer (9.9 ml) to make working solution. For 96 tests, 10 ml HRP-RBD working solution is needed.

Note: The HRP-RBD working solution should be stored at +2°C +8°C an used within 7 days. The suitable working volume should be prepared according to the number of samples. (100 µl of HRP-RBD working solution per sample).

4. Samples and Control: Add 8 µl of samples or controls in supplied 96-well dilution plate and mix with 72 µl of sample dilution buffer, with the volume ratio of 1: 9.
5. ELISA incubator or water bath must be set to +37°C ± 1°C.

Pipetting protocol



Pos.: positive control; Neg.: negative control

Manual test performance

Neutralization Reaction:

1. Pipette 80 µl 1 x HRP-RBD working solution in the separate wells of 96-well dilution plate to mix with the 80 µl of diluted samples or controls with volume ratio of 1: It's recommended that all Positive and Negative controls should be set in duplicates.
2. Cover the plate with the protective foil and use a microplate shaker to mix the reagents for 60 seconds.
3. Incubate at +37±1°C for 30 minutes.
4. Remove the protective foil and transfer 100 µl of samples/controls reaction mixture into the corresponding wells of the microplate wells.
5. Incubate at +37±1°C for 20 minutes

Washing

6. Remove the protective foil. Empty the wells and subsequently wash 4 times using 350 µl of working-strength wash buffer for each wash (automatic microplate washer is recommended to use).

Note: Leave the wash buffer in each well for 30 to 60 seconds per wash cycle. After washing, dispose of all

liquid from the microplate by tapping it on paper towel or centrifugation at 1000 g, 20 seconds to remove all residual wash buffer.

Substrate incubation:

7. Pipette 100 µl of TMB substrate solution into each well, cover the plate with protective foil and incubate for 15 minutes at +37±1°C (start timing after the addition to the last well).

Stopping:

8. Remove the protective foil and pipette 50 µl of stop solution into each wel to stop the reaction.

Measurement

9. Photometric measurement of the color intensity should be made at a wavelength of 450 nm within 5 minutes of pipetting the stop solution.

Quality Control

For each assay, both Positive and Negative controls must be included to validate the results. The OD450 of each control must meet the requirements as follows, otherwise, the test is invalid and the test should be repeated:

OD of the Negative control>0.8;

OD of the Positive control <0.2.

Evaluation

The OD of the Negative control is used to calculate the Inhibition, and the OD of Positive control is only used to evaluate the validity of the results. The inhibition of each sample can be calculated with the formulation as follows:

$$\text{Inhibition} = \left(1 - \frac{\text{OD of Sample}}{\text{(Mean OD of Negative controls)}}\right) \times 100\%$$

Interpretation of Results:

Inhibition≥20%: Positive

Inhibition<20%: Negative

Sensitivity

The cut-off value was determined at 99% sensitivity of 1735 negative sera, as < 20%.

Reproducibility

The CV ≤ 15%

Precautions

1. Read the Instruction manual carefuly before operation, and perform the test operation strictly following the instruction.
2. Avoid testing in harsh environments(such as environments containing sodium hypochlorite acid-base or acetaldehyde, and other high concentration corrosive gases and dust). Disinfection should be performed after the test.
3. After the microplate package is opened, the remaining strips should be sealed in an aluminum foil ziplock bag to prevent moisture.
4. The pipette tips cannot be repeatedly used to avold across-contamination.
5. Wash the wells gently when adding wash buffer to avoid the contamination between adjacent wells.
6. Residual liquid (>10ul) in the reagent wells after washing can interfere with the substrate and lead to false low OD readings.
7. The kit contains potential contaminants. Don't handle reagents and samples with bare hands. All samples and used kits should be considered as potentially infectious materials, the dispose of them should under local govemment and relevant national regulations.

Limitations

1. For the suspicious samples near borderline. It is recommended to re-determine and supervise dynamically.
2. Due to methodological or immuno-specific reasons, using reagents from different manufacturers to test the same sample may get different test results. Therefore, the test results of different reagents should not be directly compared with each other to avoid erroneous result interpretations. It is recommended that the laboratoryshall indicate the source of the reagents used in the test report. In continuous monitoring, if the reagent type is changed, additional continuity testing should be performed and parallel compari- son with the original reagent results to re-determine the baseline value.
3. Cross-contamination, microbial contamination severe hemolysis, or turbid samples may cause incorect results, try to avoid using such samples.