MAXLISA AntiCOVID19 neutralization antibody ELISA

1. Intended Use:
Maxlisa The Anti-SARS-CoV-2 Neutralizing Antibody ELISA Kit is an enzyme-linked immunosorbent assay intended for the qualitative detection of neutralizing antibodies against SARS-CoV-2 in human serum or plasma

2. Summary and Explanation:
coronavirus disease 2019 (COVID-19), which is a positive-sense single-stranded RNA virus that belongs to the family of coronaviruses. The most common symptoms of COVID-19 are fever, coughing, and breathing difficulties. The incubation period of COVID-19 commonly ranges from 2 to 14 days, with an avetrage of 5 days, although there also have few cases with 24 days incubation periods. Normally, the time from infection onset to onset of symptoms is one week. Coronaviruses encode four major structural proteins, spike (S), membrane (M), envelop (E), and nucleocapsid(N), notably, 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 of SARS-CoV-2 is responsible for recognizing and interacting with the cell surface receptor, angiotensin-converting enzyme-2 (ACE2). In the respiratory tract, ACE2 is widely expressed on the cell surface of alveoli, trachea, bronchi, macrophages, etc. Following the binding of the RBD to the receptor ACE2, SARS-CoV-2 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.

3. Test Principle:
The Anti-SARS-CoV-2 Neutralizing Antibody ELISA Kit is a blocking 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 and mix with horseradish peroxidase (HRP)-labelled recombinant SARS-CoV-2 RBD protein (HRP-RBD) outside of hACE2 coated ELISA plate, neutralizing antibodies in positive samples 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 that bound to SARS-CoV-2 RBD. The final solution can be read by a microplate reader at wavelength of 450nm, and the absorbance of the samples is inversely related to the inhibition% of the active neutralizing antibodies, and the inhibition% can be calculated according to the OD450 value of samples and negative control.

4. Key Contents:
Store all components at 2-8°C when not in use.

Material	96 Tests
Plate Coated with Streptavidin (1 x 96 well microplate)	Microplate coated with streptavidin, packed in a pouch with desiccant.
Positive Control (1 x 0.5 ml)	Ready to use
Negative Control (1 x 1 ml)	Ready to use
Sample Diluent (1 x 15 ml)	Buffer solution containing stabilizing proteins and preservatives
Conjugate diluent (1 x 12 ml)	Buffer solution containing stabilizing proteins and preservatives
Conjugate concentrate (100X) (1 x 0.2 ml)	RBD -HRP conjugate to be diluted with conjugate diluent.
Biotin solution (12 ml)	Ready to Use
Wash Buffer (20X) (1 x 30 ml)	Buffer containing surfactants
TMB Diluent (1 x 10 ml)	Buffer solution containing H_2O_2 with preservative.
TMB Substrate (1 x 10 ml)	To be diluted in TMB diluent before use.
Stop solution (1 x 15 ml)	Ready to use, 0.1N Sulfuric acid
Pack insert	

5. Materials required but not provided:
- Distilled or Deionized water.
- Micropipettes and Micro tips.
- Graduated cylinders for reagent bottles.
- Paper towels or Absorbent tissue.
- 70% Isopropanol solution.
- Vortex mixer.
- Incubator (37°C).
- ELISA Washer.
- ELISA Reader.
- Timer.
- Biohazard waste container with sodium hypochlorite solution.
- Disposable gloves.

6. Sample collection, Preparation and Storage:
- Only human serum or plasma samples should be used for the test.
- While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis.
- Fresh serum/plasma samples are preferred.
- Serum and plasma (EDTA) samples may be stored for up to 7 days at 2-8°C or at least 6 months as frozen (-20 to -70°C).
- Avoid repeated freezing and thawing.
- Do not use sodium azide as preservative because it inactivates horseradish peroxidase.
- Microbial contaminated and hemolyzed samples may give erroneous results.

7. Precautions:
- For in vitro diagnostic use only
- Bring all reagents and specimen to room temperature before use.
- The use of disposable gloves and proper biohazards clothing is strongly recommended while running the test.
- Do not eat, drink or smoke in the area where testing is done
- In case there is a cut/wound in hand, do not perform the test.
- Do not pipette any material by mouth.
- Do not mix components of one kit with another.
- Do not allow liquid from one well to mix with other wells.
- Do not let the strips dry in between the steps.
- All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
- Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
- Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.

8. Preparation of Reagents:
Note: Before use, allow reagents and samples at room temperature (20-30°C).
8.1. Ready for use reagents:
8.1.1. Microplate:
Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at ±2-8°C.

Caution: Handle Microwell strips with care. Do not touch the bottom exterior surface of the walls.

8.1.2. Negative control
8.1.3. Positive control
8.1.5. Sample diluent
8.1.6. Stop solution
8.2. Reagents to reconstitute:

- Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals dissolve.
- Dilute 1:20 in distilled water to obtain the ready to use washing solution. Mix 20 ml of 25X wash buffer concentrate with 480ml of distilled or deionized water. Working wash buffer is stable for 2 months when stored at 2-8°C

8.2.2. Preparation of working conjugate:
Make a 1:100 dilution of conjugate concentrate with conjugate diluent. Do not store working conjugate. Prepare conjugate 10 minutes before use.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
Enzyme Conjugate Concentrate (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Conjugate Diluent in (ml)	1	2	3	4	5	6	7	8	9	10	11	12

8.2.4. Preparation of Substrate:
Mix TMB Substrate and TMB Diluent in1:1 ratio to prepare working substrate buffer 5 to 10 minutes before use. Avoid exposure to light. Substrate should be used only after thawing at 37°C, if crystallized.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate (ml)	0.5	1	1.5	2.	2.5	3	3.5	4	4.5	5	5.5	6
TMB Diluent (ml)	0.5	1	1.5	2.	2.5	3	3.5	4	4.5	5	5.5	16

9. Test procedure:
- Bring all the reagents and specimen to room temperature before use.
- Take the required number of strips and fix them to frame and immediately close the pouch.
- Prepare template in data sheet indicating the location of controls and specimens.
- Add 50 µl of sample diluent to required number of wells
- Add 10µl of negative control A1 & B1 wells respectively.
- Add 10µl of positive control in C1
- Add 10µl of test samples in each well, starting from E1.
- Add 100 µl of working Conjugate in each well
- Mix gently and incubate at 37°C for 30 minutes
- After 30 minute add 100 µl Biotin solution in each well without discarding the previous solution, Now plate should contain (Sample diluent+sample+Conjugate+Biotin solution)
- Mix gently and incubate at 37°C for 30 minutes
- Before 5 to 10 minutes of incubation, make a 1:20 wash buffer with distilled water.
- After incubation, aspirate the contents from all the wells and wash each well 5 times with by filling approximately 300µl diluted wash buffer.
- Invert the plate and tap it on absorbent paper to remove the remaining washing solution, and then pipette 100µl of prepared diluted conjugate into each well.
- Incubate the plate at 37°C for 15 minutes.
- Add 100µl of stop solution each well.
- Read absorbance at 450nm/620 nm within 10 minutes in ELISA READER

10. Flow chart for Test Run criteria:

Add 50µl of samples diluent in each wells
↓
Add 10µl of negative, positive controls and samples
↓
Add 100µl of conjugate in each well and incubate at 37°C for 30mins
↓
Add 100µl of biotin solution in each well and incubate at 37°C for 30mins
↓
Wash each well 5 times with 300µl of wash buffer and dry with absorbent paper
↓
Add 100µl of substrate solution in each well and incubate at 37°C for 30mins
↓
Add 100µl of stop solution to stop reaction
↓
Read results at 450/620nm

11. Calculation of the cut-off value
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:

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

Abbrevations:
NC - Absorbance of Negative control
NCx - Mean Negative control
PC - Absorbance of the Positive control

Interpretation of Results:
≥20%: Positive
<20%: Negative

Quality Control
OD of the Negative Control >0.8
OD of the Positive Control <0.2

References:

- Centers for Disease Control, Update on Acquired Immune Deficiency Syndrome (AIDS) MMWR 1982; 31: 507-508.
- Chenjia Yuan, Shi Jinsong , Qiudong An , Liu Chang , Li Xin , Qiang , Ruanji Shou , mountains . Wuhan 2019 Bioinformatics coronavirus genome analysis [J / OL]. Bioinformatics : 1-10 [2020-02-10].

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