SARS-CoV-2 Neutralizing Antibody ELISA Test Kit (96 Tests)

Catalog Number: 70001
Version: 1.0

1. Intended Use

This SARS-CoV-2 Neutralizing Antibody ELISA Test Kit is intended to use for the quantitative determination of neutralizing antibodies against SARS-CoV-2 present in human serum or plasma.. It is an easy and fast in vitro assay using double antigen Sandwich enzyme linked immunosorbent assay method.

2. Introduction

SARS-CoV-2 is one of RNA viruses that cause significant disease in human such as severe acute respiratory syndrome (SARS) and other respiratory infections, as well as a variety of respiratory, gastrointestinal and other infections in an increasingly large variety of mammals and birds. [1]

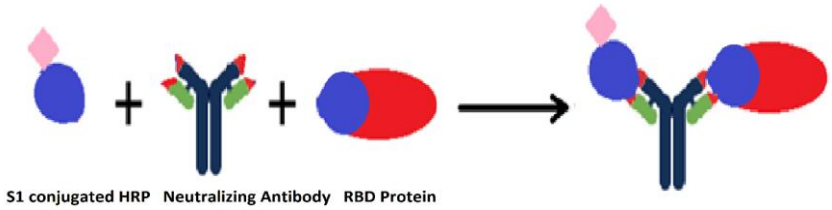
This viruses contains 4 structural proteins, including Envelope (E), Membrane (M), Nucleocapsid (N) and Spike (S). Spike protein is a transmembrane protein with two subunits S1 and S2. The S1 subunit contains a receptor binding domain (RBD), which binds to the cell surface receptor Angiotensin-Converting Enzyme 2 (ACE2) present at the surface of epithelial cells, causing mainly infection of human respiratory cells [2].

Neutralizing antibody will appear after SARS-CoV-2 infection and vaccination, and are maintained for several months.[3,4,5,6] The Neutralizing antibody detection can help us to better understand SARS-CoV-2, including how the human body responds to the virus, and the probability that the virus will causes symptomatic infections, it can also help us to estimate the number of people in area who may had suffered from SARS-CoV-2. These tests are important for public health.

This test is a 30-minute, one incubation protocol to detect SARS-CoV-2 neutralizing antibody developed in human serum. Conjunction with symptom, this test kit can help to judge this person once infected by the Covid 19 virus. It also can be use to estimate the patient Covid 19 neutralizing antibody level after vaccination.

3. Principle

The SARS-CoV-2 Neutralizing Antibody ELISA Test kit utilizes double antigen sandwich assay to detect the SARS-CoV-2 Neutralizing Antibody presented in serum. A recombinant RBD protein is coated on the 96 well plate. A S1 recombinant protein is coupled with HRP as detector. The target neutralizing antibody will bind one site with S1 and the other side with RBD to form a sandwich complex binding in the microtiter well. After a 30-minute incubation at room temperature, the wells are washed with washing solution to remove unbound substance. A TMB (Tetramethyl-benzidine) Substrate is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped by adding Stop Solution. The color will change to yellow. The amount of SARS-CoV-2 Neutralizing Antibody is directly proportional to the color intensity. Absorbance is measured spectrophotometrically at 450 nm.



The amount of SARS-CoV-2 Neutralizing Antibody can be calculated as BAU/ml (Binding Antibody Unit/ml) based on the Standard Curve.

Internal Standard for Anti – Neutralizing Antibody is a polyclonal antibody which was calibrated to the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human): NIBSC code:20/136

4. Materials provided with the kit

(1) Antigen-Coated Wells (1 plate, 96 wells)
Microtiter wells coated with recombinant RBD Protein
(2) S1—HRP conjugate solution (12 mL)
(3) Calibration Set (2-point, plus one for zero)
Calibrator 0: zero, 0.3 ml
Calibrator 1: 400 BAU/ml, 0.2 ml
Calibrator 2: 800 BAU/ml,0.2 ml
(4) Washing Solution (120 mL)
(5) TMB Reagent (1 bottle, 11 mL/bottle)
Contains one-step TMB solution
(6) Stop Solution (1 bottle, 11 mL/bottle)
Contains diluted H_2SO_4

5. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture if stored at 2°C to 8°C, and the opened kit is stable for up to 1 month after opening and store at 2°C to 8°C.

6. Materials Required But Not Provided

(1) Test tubes and beakers
(2) Adjustable pipettes (20-1000 µL)
(3) Timer
(4) Vortex mixer or equivalent.
(5) Absorbent paper or paper towels
(6) Graph paper
(7) Microtiter plate reader

7. Warnings and Precautions

- For professional *in vitro* research test use only.
- Do not use the product beyond the expiration date.
- Handle all specimens as potentially infectious.

- Follow standard laboratory procedure and biosafety guidelines for the handling and disposal of potentially infectious materials.
- When the assay procedure is completed, dispose specimens after autoclaving at 121º C for at least 20 min or treating with 0.5% Sodium Hypochlorite for 1-2 hours.

8. Collection

- Handle all the serum sample as if capable of transmitting infectious agents.
- The NCCLS provides recommendations for handling and storing serum specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).

9. Assay Protocol

1) For obtaining accurate results, 2 duplicate of each calibrator is recommended.
2) Add 20 ul of samples or internal standard (calibrator) into each well.
3) Add 100 ul of RBD-Fc – HRP to each well.
4) Mix well and cover the plate with plate sealer.
5) Incubate at RT for 30 minutes.
6) Remove the plate sealer and wash the plate with 300 ul of Washing Solution for 4 times.
7) Pat the plate on the paper towel to remove residual liquid in the well after each washing step.
8) Added 100 ul of TMB substrate into each well and stay at room temperature in dark for 15 minute.
9) Added 100 ul of stopping solution into each well, mix well and immediately read the result via EIA Reader.

10. Calculation of Results

(1) Calculate the mean absorbance value (OD_{450}) for each set of internal standards, and samples.

(2) Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in IU/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

(3) Using the mean absorbance value for each sample, determine the corresponding concentration of SARS-CoV-2 Neutralizing Antibodies (BAU/ml) from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

(4) If the measuring is a diluted patient sample, the obtained values of the patient samples should be multiplied by the dilution factor.

11. Performance Characteristics

(1) Internal Standard Curve for SARS-CoV-2 NAbs

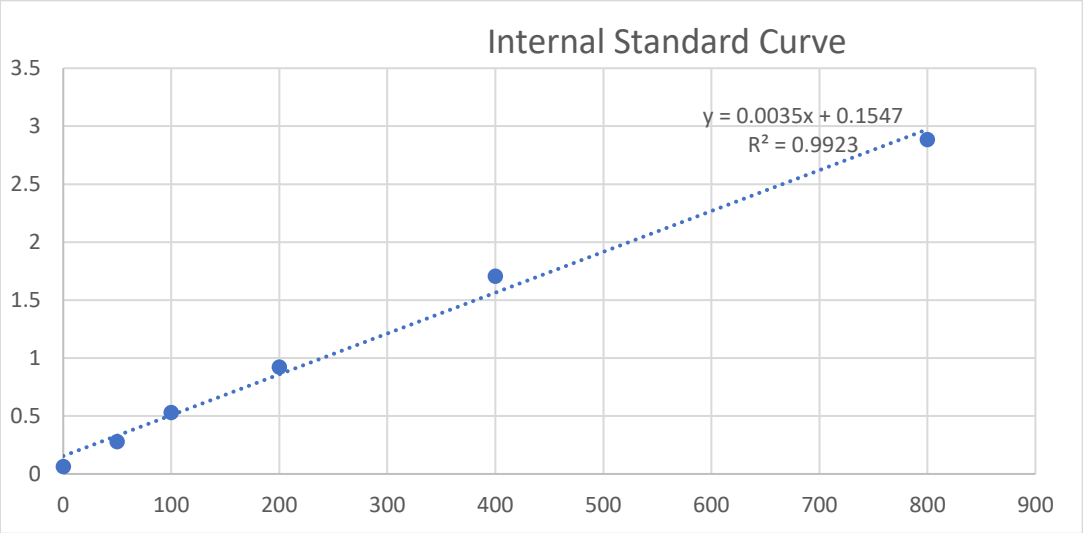


Figure 1: Example of internal standard curve for SARS-CoV-2 NAbs

	Internal Standard, *BAU/ml					
	0	50	100	200	400	800
OD_{450}	0.064	0.279	0.530	0.927	1.708	2.886

* BAU: Binding Antibody Unit

(2) Sensitivity and specificity

		Abbott Architect SARS-COV-2 IgG	
		Positive	Negative
LQ SARS-COV-2 Neutralizing Antibody EIA Kit	Positive	43	0
	Negative	0	75

Sensitivity = 43/43 = 100%

Specificity = 75/75 = 100%

100% sensitivity and specificity by comparing to Abbott Architects IgG data.

(3) Repeatability Study

For each concentration of SARS-CoV-2 Neutralizing Antibody, five duplicates were run in the same day same running. Mean, Standard deviation (SD) and CV were calculated.

	Internal Neutralizing Antibody, ug/mL					
	12.5	25	50	100	200	400
n	10	10	10	10	10	10
Mean	0.066	0.110	0.222	0.432	0.880	1.704
STDEV	0.006919	0.005021	0.009295	0.016956	0.053906	0.10626
CV	10%	4.5%	4.1%	3.9%	6.1%	6.2%

Conclusion: The CV of 6 analytes concentration all under 20% which is the criteria for CV in ELISA Test.

(4) Reproducibility Study

In 5 days, a duplicate of each concentration of SARS-CoV-2 Neutralizing Antibody was run. Mean, Standard deviation (SD) and CV were calculated.

	Internal Neutralizing Antibody, ug/mL					
	12.5	25	50	100	200	400
n	10	10	10	10	10	10
Mean(Five days)	0.0882	0.129	0.2577	0.4839	0.9439	1.814
STDEV	0.017687	0.020105	0.029082	0.041165	0.075919	0.129677
CV	20.01%	15.5%	11.2%	8.50%	8.10%	7.10%

Conclusion: The CV of 12.5 ug/mL (20.01%) was higher than the criteria of 20%. The others were all in acceptable range, under 20%.

(5) Accuracy Study

Estimate the accuracy at each sample concentration by dividing mean of all result obtained by the concentration of added Internal SARS-CoV-2 Neutralizing Antibody and multiplying by 100.

	Internal SARS-CoV-2 Antibody, ug/mL					
	12.5	25	50	100	200	400
mean	0.066	0.110	0.222	0.432	0.880	1.704
	Analyte (Positive Antibody), ug/mL					
X = (Y – 0.0151)/0.0043	11.8	22.06	48.1	96.9	201.1	392.7
Expected Value	12.5	25	50	100	200	400
Accuracy	94.4%	88.2%	96.2%	96.9%	100.5%	98.1%

Conclusion: The accuracy at each concentration all higher than 80%.

(6) LOD

	Internal SARS-CoV-2 Antibody, ug/ml					
	12.5	**25**	50	100	200	400
CV of Repeatability	10%	**4.5%**	4.1%	3.9%	6.1%	6.2%
CV of Reproducibility	20.1%	**15.5%**	11.2%	8.5%	8.1%	7.1%
Accuracy	94.4%	**88.2%**	96.2%	96.9%	100.5%	98.1%

Conclusion: [a] Bold values indicate the column for the lowest concentration of analyte that provides CVs of Repeatability and Reproducibility less than 20% and accuracy in the range of 85% - 115%.

The LOD is 25 ug/ml, or 20 BAU/ml (in house calibration data: 100 BAU/ml = 150 ug/ml of internal antibody)

Cutoff will set up at 0.13 (mean + 3SD) at OD_{450}.

(7) Positive Patient Serum Dilution Testing

The following was a typical test result of a diluted serum from Stanford Blood Center.

Patient # 90417

Dilution	**Not diluted**	**1:10**	**1:100**	**1:1000**	**1:2000**	**1:4000**	**1:8000**
OD_{450}	4.044	3.338	0.735	0.149	0.086	0.073	0.067

12. References

[1] Suthar MS, Zimmerman MG, Kauffman RC, et al. Rapid generation of neutralizing antibody responses in COVID-19 patients. *Cell Rep Med*. 2020;1(3):100040. doi:10.1016/j.xcrm.2020.100040PubMedGoogle Scholar

[2] Anshumali Mittal1 *, Kavyashree Manjunath2 , et al., R COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2 Anshumali Mittal1 *, Kavyashree Manjunath2 , Rajesh Kumar RanjanID3 , Sandeep KaushikID4 , Sujeet Kumar5 , Vikash VermaID6 *PLOS Pathogens | https://doi.org/10.1371/journal.ppat.1008762 August 21, 2020

[3] Jackson LA, Anderson EJ, Rouphael NG, et al; mRNA-1273 Study Group. An mRNA vaccine against SARS-CoV-2. *N Engl J Med*. 2020;383(20):1920-1931. doi:10.1056/NEJMoa2022483PubMedGoogle ScholarCrossref

[4] Widge AT, Rouphael NG, Jackson LA, et al; mRNA-1273 Study Group. Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. *N Engl J Med*. 2021;384(1):80-82. doi:10.1056/NEJMc2032195PubMedGoogle ScholarCrossref

[5] Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science*. 2021;371(6529):eabf4063. doi:10.1126/science.abf4063PubMedGoogle Scholar

[6] Widge AT, Rouphael NG, Jackson LA, et al; mRNA-1273 Study Group. Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. *N Engl J Med*. 2021;384(1):80-82. doi:10.1056/NEJMc2032195PubMedGoogle ScholarCrossref

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