Life Technologies™
Your Molecular & Cell Technology Partner

# *LTI* Quantitative SARS-CoV-2 Neutralizing Antibody ELISA Kit

*Enzyme Linked Immunosorbent Assay (ELISA) for the quantitative detection of the SARS-CoV-2 neutralizing antibody concentration in a human serum.*

REF LT6031K RUO

## INTENDED USE

The LTI quantitative SARS-CoV-2 neutralizing antibody ELISA Kit is an Enzyme-Linked Immunosorbent Assay (ELISA) kit intended for the *quantitative* measurement of the neutralizing antibody to SARS-CoV-2 receptor binding domain (RBD) of its spike protein in human serum. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. 263a, to perform moderate or high complexity tests. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, as well as in the convalescent stage. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities. The LTI quantitative SARS-CoV-2 neutralizing antibody ELISA Kit may shows false higher antibody concentration due to cross-reactivity from pre-existing antibody or other possible causes.

**For Research Use Only. Not for use in Diagnostic Procedures.**

## SUMMARY OF PHYSIOLOGY

2019 novel coronavirus (2019-nCoV or SARS-CoV-2 or COVID-19) is a single-stranded RNA coronavirus[2]. Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and bat coronaviruses[7]. In humans, coronaviruses cause respiratory infections[3]. Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) [4]. Results suggest that the spike protein retains sufficient affinity to the Angiotensin Converting Enzyme-2 receptor to use it as a mechanism of cell entry[6]. Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing[1]. IgG is the most abundantly found immunoglobulin to be produced in response to an antigen and will be maintained in the body after initial exposure for long term response[5].

## ASSAY PRINCIPLE

This ELISA kit is designed, developed, and produced for the *quantitative* measurement of the neutralizing antibodies to SARS-CoV-2 RBD of its spike protein in human serum This assay utilizes the microplate-based enzyme immunoassay technique.

Assay calibrators, controls, and human serum samples are added to the microtiter wells of a microplate coated with streptavidin. Simultaneously, horseradish peroxidase (HRP) labeled COVID-19 recombinant spike protein and biotinylated angiotensin converting enzyme-2 (ACE-2) are added to each well. After the first incubation period, the unbound protein matrix is removed with a subsequent washing step; a complex of "Streptavidin---Biotin-ACE2---HRP-COVID-19 recombinant spike protein" is formed. If there is specific COVID-19 neutralizing antibody present in the tested specimen, the formation of the above complex is blocked. A color reaction with a substrate solution in a timed reaction is measured in a spectrophotometric microplate reader. The HRP enzymatic activity of the complex on the wall of the microtiter well is inversely proportional to the amount of the COVID-19 neutralizing antibody level in the tested specimen.

## REAGENTS: PREPARATION AND STORAGE

The test kit must be stored at 2 – 8°C. Refer the label on a kit box for the expiration date. All components are stable until expiration date.

### 1. Streptavidin Coated Microplate

Microplate coated with streptavidin.

Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to use

### 2. Biotinylated ACE2

Biotinylated recombinant ACE2 protein.

Qty: 1 x 5 mL
Storage: 2 – 8°C
Preparation: Ready to use

### 3. HRP labeled Spike Protein

HRP labeled spike protein in a stabilized protein matrix.

Qty: 1 x 5 mL
Storage: 2 – 8°C
Preparation: Ready to use

### 4. ELISA Wash Concentrate

Surfactant in a phosphate buffered saline with non-azide reservative.

Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30x Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use

### 5. ELISA HRP Substrate

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 15 mL
Storage: 2 – 8°C
Preparation: Ready to use

### 6. ELISA Stop Solution

0.5 M sulfuric acid.

Qty: 1 x 15 mL
Storage: 2 – 25°C
Preparation: Ready to use

### 7. nCoV Neutralizing Antibody Calibrator Level 1

A ready-to-use sample dilution buffer.

Qty: 1 x 15 mL
Storage: 2 – 8°C
Preparation: Ready to use

### 8. nCoV Neutralizing Antibody Calibrator Level 5

Calibrators with a bovine serum albumin based matrix with non-azide preservative. Refer to vials for exact concentration.

Qty: 1 x 0.5 mL
Storage: 2 – 8°C.
Preparation: Lyophilized powder (see Assay Procedure section)

### 9. nCoV Neutralizing Antibody Controls

Controls with a bovine serum albumin-based matrix with non-azide preservative.

Qty: 2 x 0.5 mL
Storage: 2 – 8°C.
Preparation: Lyophilized powder (see Assay Procedure section)

## SAFETY PRECAUTIONS

The reagents are for in-vitro diagnostic use only. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of

contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Keep out of reach skin, eyes and/or clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Exercise Good Laboratory Practices.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 20 µL, 100 µL, and 1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 mm glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
11. Calibrated Timer.

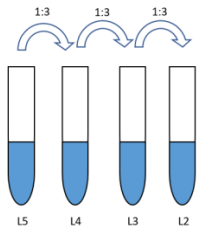
## SAMPLE COLLECTION & STORAGE

Only 50 µL of human serum is required for measurement in duplicate. Samples should only be used on the same day or stored below -20ºC. Severe hemolytic samples should not be used.

## ASSAY PROCEDURE

**1. Reagent Preparation**

1. Prior to use, allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate (10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.
3. Reconstitute nCoV Neutralizing Antibody Calibrator Level 5 (31285) by adding 0.5 mL deionized water.
4. Prepare calibrator level 2, 3 and 4by 1:3 serial dilutions of level 5 (31285) with nCoV Neutralizing Antibody Calibrator Level 1 (31281). Assay calibrators should be used within 2 hours and should be stored below -20°C. Do not exceed 3 freeze-thaw cycles. The calibrator concentrations are indicated in the certificate of analysis of the kit.




**2. Assay Procedure**

1. Place a sufficient number of microwell strips (10040) in a holder to run the calibrators, controls, and samples in duplicate.
2. Test Configuration

| Row | Strip 1 | Strip 2 | Strip 3 |
|---|---|---|---|
| A | Calibrator level 1 | Calibrator level 5 | Sample 2 |
| B | Calibrator level 1 | Calibrator level 5 | Sample 2 |
| C | Calibrator level 2 | Control 1 | Sample 3 |
| D | Calibrator level 2 | Control 1 | Sample 3 |
| E | Calibrator level 3 | Control 2 | Sample 4 |
| F | Calibrator level 3 | Control 2 | Sample 4 |
| G | Calibrator level 4 | Sample 1 | Sample 5 |
| H | Calibrator level 4 | Sample 1 | Sample 5 |

3. Add **25µL** of calibrators, controls, and unknownsamples into the designated microwells.
4. Add **50 µL**of HRP labeled spike protein (31279) into each microwell.
5. Add **50 µL**of biotinylated ACE2 (31278) into each microwell.
6. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25ºC)**for**45 minutes.**
7. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of the substrate (10020) into the microwells.
9. Mix gently and cover the plate with aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
10. Remove the aluminum foil and add **100 µL** of stop solution (10030) into each of the microwells. Mix by gently by tapping the plate.
11. Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.

## PROCEDURAL NOTES

1. Calibrator L4 can be made by mixing 200 µL of calibrator L5 with 400 µL of calibrator 1. Calibrator L3 can be made by mixing 200 µL of calibrator L4 with 400 µL of calibrator 1. Calibrator L2 can be made by mixing 200 µL of calibrator L3 with 400 µL of calibrator 1.
2. It is recommended that all samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
3. Keep light-sensitive reagents in the original bottles and avoid unnecessary exposure to the light.
4. Store any unused antibody-coated strips in the foil ziploc bag with desiccant to protect from moisture.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation time(s)and/or temperature(s) other than those stated in the package insert may affect the results.
7. Avoid air bubbles in the microwell as it could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed thoroughly and gently prior to use. Avoid foaming.

## QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known SARA-CoV-2 neutralizing antibody levels. LTI recommends including own laboratory controls in addition to those provided with thekit.

## INTERPRETION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. The calibration curve is generated by the absorbance of all calibrator levels on the ordinate against the calibrator concentration on appropriate computer assisted data reduction programfor the calculation of results.
3. It is recommended to use following curve fits: (1) 4-Parameteror (2) Point-to-Point.
4. The SARS-CoV-2 neutralizing antibody concentrations for the controls and patient samples are read directly from the calibration curve using their respective absorbance.

## LIMITATIONS OF THE PROCEDURE

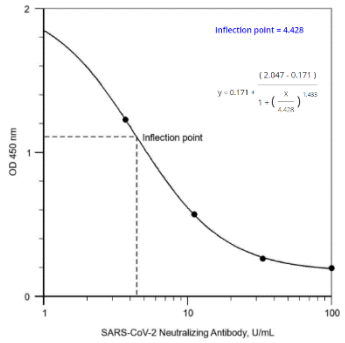
1. The values of the assay calibrators were established by diluting ahuman SARS-CoV-2 neutralizing antibodystock in a phosphate buffer protein matrix.
2. Patients with low immunity or other diseases that affect immune function, failure of critical systemic organs, and use of drugs that suppress immune function can also lead to negative results. Previous infection of SARS or other coronavirus strains may present a low-level SARS-CoV-2 neutralizing antibodydue to similarity of different strains.
3. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.
4. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
5. The LTI quantitative SARS-CoV-2neutralizing antibody ELISA Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

## EXAMPLE DATA (Calibration Curve)

This ELISA calculates the concentration values IgG of the samples and the controls by a calibration curve (fitting method: four parameters or point-to-point) and the measured absorbance.The following is a typical calibration curve:

| Well ID | OD 450 nm | Average |
|---|---|---|
| Calibrator Level 1: 0 U/mL | 2.012 | 2.047 |
| | 2.082 | |
| Calibrator Level 2: 3.7 U/mL | 1.203 | 1.228 |
| | 1.252 | |
| Calibrator Level 3: 11.1 U/mL | 0.586 | 0.570 |
| | 0.555 | |
| Calibrator Level 4: 33.5 U/mL | 0.263 | 0.263 |
| | 0.264 | |
| Calibrator Level 5: 100 U/mL | 0.196 | 0.196 |
| | 0.195 | |
| | 0.998 | |

*Note: This curve should not be used in lieu of calibrator curve run with each assay.*




## EXPECTED VALUES

One hundred donor serum samples from December 2018 to February 2019 were collected and tested. The average concentration of SARS-CoV-2 neutralizing antibody was found to be 1.13 U/mL with a median at 0.64 U/mL and standard deviation at 2.91 U/mL. The manufacturer recommended $P_{97.5}$ cut-off level is **5 U/mL** for the present of neutralizing antibody in test sample. It is highly recommended that each laboratory should establish their own cut off level based on local population.

## WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Life Technologies DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Life Technologies India Pvt Ltd be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

## REFERENCES

1. CDC (2020). Transmission of Novel Coronavirus (COVID-19).
2. 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].
3. Fehr, A. R., & Perlman, S. (2015). Coronaviruses: An Overview of Their Replication and Pathogenesis. *Coronaviruses Methods in Molecular Biology*, 1–23. doi: 10.1007/978-1-4939-2438-7_1
4. Li, F., Li, W., Farzan, M., & Harrison, S. (2005). Structure of SARS coronavirus spike receptor-binding domain complexed with its receptor. doi: 10.2210/pdb2ajf/pdb
5. Wu, L.-P., Wang, N.-C., Chang, Y.-H., Tian, X.-Y., Na, D.-Y., Zhang, L.-Y., Liang, G.-D. (2007). Duration of Antibody Responses after Severe Acute Respiratory Syndrome. Emerging Infectious Diseases, 13(10), 1562–1564. doi: 10.3201/eid1310.070576
6. Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., Hao, P. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Science China Life Sciences*. doi: 10.1007/s11427-020-1637-5
7. Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. doi: 10.1038/s41586-020-2012-7

**Contact**:
**Life Technologies (India) Pvt. Ltd.**
**306, Aggarwal City Mall, Opposite M2K Pitampura,**
**Delhi – 110034 (INDIA).**
**Ph: +91-11-42208000, 42208111, 42208222**
**Mobile: +91-9810521400**
**Fax: +91-11-42208444**
**Email: customerservice@lifetechindia.com**
**Web: www.lifetechindia.com**