Product Manual

SARS-CoV-2 Spike IgG ELISA Kit (RUO)

Catalog #: ENZ-KIT190

1 x 96 well assay

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Product Manual

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Carefully note the handling and storage conditions of each kit component.

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INTENDED USE

The SARS-CoV-2 Spike IgG ELISA Kit is designed for the qualitative measurement of SARS-CoV-2 virus IgG antibody in serum samples.

INTRODUCTION

The SARS-CoV-2 virus is a single-stranded RNA coronavirus that causes respiratory infections. IgG is an immunoglobulin that is produced in response to an antigen such as SARS-CoV-2 virus. This assay is designed for the qualitative measurement of the human SARS-CoV-2 IgG.

PRINCIPLE OF THE ASSAY

The assay controls and diluted serum samples are added to the microtiter wells of a microplate that was coated with SARS-CoV-2 S1 antigen RBD protein. After an incubation period, the wells are washed removing any unbound proteins. A horseradish peroxidase (HRP) labeled mouse anti-human IgG antibody is added to each well. The wells are subsequently washed to remove any unbound antibody. If SARS-CoV-2 IgG is present in the sample, a complex is formed between the SARS-CoV-2 S1 antigen RBD protein: human anti-SARS-CoV-2 IgG antibody: HRP labeled anti-human IgG antibody. The well is incubated with a substrate solution in a timed reaction and measured in a spectrophotometric microplate reader. The intensity of the signal is proportional to the amount of anti-SARS-CoV-2 IgG antibody level in the tested specimen.

MATERIALS PROVIDED

1. High Positive Control, 500 µL
2. Low Postitive Control, 500 µL
3. Negative Control, 500 µL
4. Sample Diluent, 15 mL
5. Wash Buffer Concentrate (20X), 50 mL
6. Stop Solution, 10 mL
7. TMB Substrate, 10 mL
8. HRP Conjugate, 10 mL
9. SARS-CoV-2 Antigen Coated Microplate
10. Plate Sealer, 2 each

REQUIRED MATERIALS NOT PROVIDED

- Deionized or distilled water
- Precision pipets for volumes between 5 µL and 1,000 µL
- Repeater pipet for dispensing 100 µL
- Disposable beakers for dilution buffer concentration
- Incubator for microtiter plate, capable of 37°C
- Adsorbent paper for blotting
- Microplate reader capable of reading at an optical density of 450nm

STORAGE

Upon receipt, store entire kit at 4°C. When stored as indicated, all components are stable until the kit's expiration date.

WARNINGS AND PRECAUTIONS

Important/ Warning

1. The SARS-CoV-2 Spike IgG ELISA Kit workflow should be performed by qualified and trained staff to avoid the risk of erroneous results.
2. Kit components should be stored at the proper temperatures as indicated on the labels.
3. Always check the expiration date prior to use of reagents. DO NOT use expired reagents.
4. When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
5. Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
6. Handle all samples and controls as if they are capable of transmitting infectious agents.
7. Some of the kit components contain hazardous substances. Consult the appropriate material safety data sheets (MSDS) for more information.
8. Observe good laboratory practices. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. Any biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

PROCEDURAL NOTES

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.

REAGENT PREPARATION

1X Wash Buffer
Prepare 1X Wash Buffer by adding 1 part of Wash Buffer Concentrate (20X) to 19 parts water.

PROCEDURES

Note: Bring all reagents to room temperature (18-25°C) before use. Refer to the Assay Layout Sheet for recommended plate setup. If the kit will not be used up in a single assay, take out the necessary strips and store the remaining at the designated conditions.

1. Dilute serum samples in Sample Diluent at 1:10 ratio. For example, add 10µL of serum sample and add to 90µL of Sample Diluent.
2. Leave the denoted duplicate Blank wells empty.
3. Add 100µL of Sample Diluent in duplicate to the No Sample Control (S0) wells.
4. Add 100µL of High Positive Control in duplicate to the appropriate wells.
5. Add 100µL of Low Positive control in duplicate to the appropriate wells.
6. Add 100µL of Negative control in duplicate to the appropriate wells.
7. Add 100µL of diluted samples to remaining empty wells, except for the Blank wells.
8. Seal plate and incubate plate at 37°C for 30 minutes.
9. Empty or aspirate the contents of the wells and add 350µL 1X Wash Buffer to each well.
10. Immediately empty or aspirate the contents of the wells and repeat the wash steps three more times (for a total of 4 washes).

Note: Be sure to completely remove excess wash buffer (e.g. firmly tap the plate on a lint-free paper towel)

11. Add 100µL of HRP Conjugate to each well except the Blank wells.
12. Seal plate and incubate at 37°C for 15 minutes.
13. Empty or aspirate the contents of the wells and add 350µL 1X Wash Buffer.
14. Immediately empty or aspirate the contents of the wells and repeat the wash four more times (for a total of 5 washes).
    Note: Be sure to completely remove excess wash buffer (e.g. firmly tap the plate on a lint-free paper towel)
15. Add 100µL TMB Substrate to all wells and incubate at 37°C temperature for 15 minutes in the dark.
16. Add 50µL Stop Solution to complete the reaction.
17. Read plate at an optical density of 450nm.

CALCULATION OF RESULTS

Calculation of Net OD Values

The average OD of the two blank wells is subtracted from all OD values for all wells including the Negative Control, Low Positive Control, High Positive Control, and Samples.

$$\text{Net Sample OD} = \text{Sample OD} - \text{Average Blank OD}$$

Cut-off Value (COV)

Cut-off Values was experimentally determined to be 0.08.

Index Value

Data comparison between different assay runs is facilitated by using an index value whereby sample absorbance is expressed relative to the assay Cut-off Value (COV). A sample's index value is calculated using the formula:

$$\text{Index Value} = \frac{\text{Net Sample OD}}{\text{Cut-off Value (COV)}}$$

Interpretation of Results

The presence or absence of anti-SARS-CoV-2 virus IgG is determined in relation to the calculated Cut-off Value (COV).

Negative Specimens	Index ≤ 1.0
Positive Specimens	Index >1.0

Clinical Interpretation

Negative: No detection of the SARS-CoV-2 Spike protein IgG. Implies no past infection.

Positive: Detection of the SARS-CoV-2 Spike protein IgG. Implies past exposure to SARS-CoV-2.

QUALITY CONTROL

The Low Positive and Negative Controls must always be included to determine the validity of test results. The Low Positive Control OD should be greater than the COV to ensure the assay cut-off is valid. The negative control guards against false positive results and should always be less than the COV.

Results from an assay are considered valid if the following criteria are met:

- The mean absorbance of the High Positive Control ≥ 0.50 Optical Density Units.
- The mean absorbance of the Low Positive Control ≥ 0.10 Optical Density Units.
- The mean absorbance of the Negative Control ≤ 0.08 Optical Density Units.

Controls Out of Range

If any of the above criteria is not met, the assay is considered invalid. The run must be repeated.

PERFORMANCE CHARACTERISTICS

Summary

The clinical utility of the SARS-CoV-2 Spike IgG ELISA Kit was assessed in a population group of 149 specimens (combination of male and female) conducted at Enzo Life Sciences, Inc, Farmingdale, NY, USA using freshly collected specimens. As there is no standard reference method for SARS-CoV-2 IgG serology, samples were analyzed using the AMPIPROBE® SARS-CoV-2 RT-PCR Assay Kit as a reference.

Table 1: Comparison of the SARS-CoV-2 Spike IgG ELISA Kit results to the diagnosis based on the AMPIPROBE® SARS-CoV-2 RT-PCR Assay Kit as a reference.

		RT-PCR	
		Positive	**Negative**
IgG ELISA	**Positive**	36	4
	Negative	0	109

Specificity = 96.5%
Sensitivity = 100%

Intra-assay Reproducibility

Intra-assay reproducibility data is presented below (**Table 2**). The same results are presented in terms of index value in **Table 3.**

Table 2: Intra-assay reproducibility expressed in terms of OD on 15 replicates of each provided controls.

Control	Mean OD	SD	%CV	n
High Positive Control	0.770	0.080	10	15
Low Positive Control	0.166	0.006	3	15
Negative Control	0.068	0.002	3	15

Table 3: Intra-assay reproducibility expressed in terms of index values on 15 replicates of each provided controls.

Control	Index Value	SD	%CV	n
High Positive Control	9.625	0.998	10	15
Low Positive Control	2.075	0.070	3	15
Negative Control	0.850	0.029	3	15

Inter-assay Reproducibility

Inter-assay reproducibility data is presented below (**Table 4**). The same results are presented in terms of index value in **Table 5.**

Table 4: Inter-assay reproducibility expressed in terms of OD on 15 replicates of each provided controls.

Control	Mean OD	SD	%CV	n
High Positive Control	0.668	0.102	15	15
Low Positive Control	0.163	0.033	20	15
Negative Control	0.049	0.017	34	15

Table 5: Inter-assay reproducibility expressed in terms of index values on 15 replicates of each provided controls.

Control	Index Value	SD	%CV	n
High Positive Control	8.355	1.293	15	15
Low Positive Control	2.031	0.473	23	15
Negative Control	0.618	0.473	39	15

Reproducibility and Robustness

The Reproducibility and Robustness is determined by the value of the Z-Factor. The Z-Factor is defined as:

$$\text{Z-Factor} = 1-((3x(SD_{pos} + SD_{neg}))/|Mean_{pos}-Mean_{neg}|)$$

If $0.5 \leq$ Z-Factor < 1.0, then the assay is an excellent assay; a Z-Factor of 0.5 denotes 12 standard deviations between the $Mean_{pos}$ and $Mean_{neg}$.

If $0 <$ Z-Factor < 0.5, then the assay is a marginal assay.

If the Z-Factor = 0, then the assay is invalid.

The Z-Factor values for each assay day (Intra-Assay) and for all the assayed data combined (Inter-Assay) are given in **Table 6.**

Table 6: Z-Factors for each of the Intra-Assay and Inter-Assay data sets.

Assay	Z-factor
Intra-Day 1	0.65
Intra-Day 2	0.80
Intra-Day 3	0.72
Inter-Assay	0.50

PRECISION

Intra-Assay: ≤ 15% CV of optical density
Inter-Assay: ≤ 20% CV of optical density

ANALYTICAL SPECIFICITY

Cross reactivity was determined by using 77 serum samples collected prior to December 2019. The 77 samples were tested for the detection of SARS-CoV-2 IgG. Of the 77 samples, 73 produced negative results and four produced a positive result. The 77 samples are presumed negative for SARS-CoV-2, but were not tested by a comparator method. The sample optical density values at 450nm were plotted from largest to smallest. The observed cross reactivity from the 77 samples tested demonstrated 95% specificity.

ANALYTICAL SENSITIVITY

In order to examine the analytical sensitivity of the assay, the amount of signal detected in the no sample control wells (S0) was experimentally determined (n=29). The analytical sensitivity is given as 2X the standard deviation which was calculated to be 520 pg/mL.

NOTES

NOTES

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Product Number: *ENZ-KIT190* Rev 111920