

# ErbaLisa COVID-19 IgM

Catalog No. IME00137 (96 Tests)

## INTENDED USE

The ErbaLisa COVID-19 IgM kit is intended for the detection of IgM antibodies to SARS-CoV-2 (COVID-19) in human serum. The ErbaLisa COVID-19 IgM ELISA should be employed in conjunction with the ErbaLisa COVID-19 IgG ELISA test kit, for complete differential information on the antibody status.

## SUMMARY AND EXPLANATION

Since late December 2019, an outbreak of a novel coronavirus disease (COVID-19; previously known as 2019-nCoV)<sup>1</sup>, was first reported in Wuhan, China,<sup>2</sup> which has subsequently spread globally in a short period of time. COVID-19 or SARS-CoV-2 has demonstrated the capability to spread rapidly, leading to significant impacts on healthcare systems and causing societal disruption and the potential public health threat posed by COVID-19 is high<sup>3</sup>. In general, COVID-19 is an acute resolved disease, but it can also be deadly, with a 2% case fatality rate. COVID-19 is a single-stranded RNA coronavirus<sup>4</sup>. The viral infection causes a series of respiratory illness including severe respiratory syndrome, indicating the virus most likely infects respiratory epithelial cells and spreads mainly via respiratory tract from human to human<sup>4</sup>. Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N)<sup>5</sup>. Virus infections are characterized by elevations in specific IgM antibody levels 3 to 5 days after the onset of symptoms; this generally persists for 30 to 60 days. IgG levels also become elevated after 10 to 14 days.

## PRINCIPLE OF THE TEST

Diluted patient serum is added to wells coated with purified COVID-19 antigen. SARS-CoV-2 IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away, and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

MATERIALS PROVIDED		96 Tests
1.	Microwells coated with COVID-19 Antigen	12x8x1
2.	Sample Diluent: 1 bottle (ready to use)	22 ml
3.	Calibrator: Yellow Cap, 1 vial (ready to use)	1ml
4.	Positive Control: Red Cap, 1 vial (ready to use)	1ml
5.	Negative Control: Blue Cap, 1 vial (ready to use)	1ml
6.	Enzyme conjugate: 1 bottle (ready to use)	13ml
7.	TMB Substrate: 1 bottle (ready to use)	13ml
8.	Stop Solution: 1 bottle (ready to use)	13ml
9.	Wash concentrate 20X: 2 bottles	2x25ml

## MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. precision pipettes
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

## STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

## WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas where specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

5. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

### SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.
3. Warning: Do not use IgM Sample Diluent for IgG testing.

### REAGENT PREPARATION

Before running the test, prepare the following:

**Wash Buffer:** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (Average of 23°C).

### ASSAY PROCEDURE

***All reagents and specimens must be allowed to come to room temperature before use and must be GENTLY mixed without foaming. Once the procedure has started, all steps should be completed without interruption.***

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control and positive control are ready to use and are recommended to be assayed in duplicate. Calibrator is ready to use and is compulsory to be assayed in duplicate. Prepare 1:21 dilution of test samples, by adding 10µl of the sample to 200µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells six times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells six times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µL of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 10 min. A dual wavelength is recommended with reference filter of 600-650 nm.

### CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the mean values of each sample by cut-off value.

### EXAMPLE OF TYPICAL RESULTS

Calibrator mean OD = 0.8

Calibrator Factor (CF) = 0.5

Cut-off Value =  $0.8 \times 0.5 = 0.400$

Positive Control OD = 1.2

Ab Index =  $1.2 / 0.4 = 3$

Patient Sample OD = 1.6

Ab Index =  $1.6 / 0.4 = 4.0$

### QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The OD of the Calibrator should be greater than 0.250
2. The Ab Index for Negative control should be less than 0.9
3. The Ab Index for Positive control should fall within the range specified on the COA/Label.

### INTERPRETATION

The following is intended as a guide to interpretation of COVID-19 IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

### ANTIBODY INDEX INTERPRETATION

- < 0.9 No detectable IgM Antibody to COVID-19.
- 0.9 - 1.1 Borderline Positive. Follow-up testing is recommended if clinically indicated.
- > 1.1 Detectable IgM antibody to COVID-19.

## LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.
3. This test is only provided for use by clinical and not for at home testing.
4. This test has not been reviewed by the FDA.
5. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
6. Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
7. Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

## PERFORMANCE CHARACTERISTICS

### 1. Sensitivity & Specificity

30 serum samples collected from previously RT-PCR confirmed COVID-19 patients were tested. 50 Normal healthy patients with samples collected before COVID-19 outbreak (prior to December 2019) were tested. The results are as follows:

	Test Positive	Test Negative
Confirmed Positive	30	0
Confirmed Negative	5	45

The Sensitivity is 100%

The Specificity is 90%

### 2. Class Specificity

This assay does not show any cross reaction to IgG.

### 3. Interference

The following interferents were tested and no influence was observed on the sample results up to the concentrations indicated below:

- Haemoglobin (10 mg/mL)
- Bilirubin (400ug/mL)
- Biotin (200ng/mL)

### 4. Precision

#### Intra-Assay Precision


Serum	No. of Replicates	Mean (OD 450nm)	Standard Deviation	Coefficient of Variation (%)
1	16	0.28	0.02	7.20%
2	16	0.93	0.08	8.06%
3	16	2.36	0.13	5.67%

#### Inter-Assay Precision

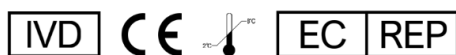
Serum	No. of Replicates	Mean (OD 450nm)	Standard Deviation	Coefficient of Variation (%)
1	16	0.26	0.02	9.42%
2	16	0.89	0.08	8.54%
3	16	2.47	0.17	6.71%

## REFERENCES:

1. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020; (published online Feb 3.)
2. Huang C Wang Y Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020; 395: 497-506
3. CDC (2020). Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency
4. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H, Evidence for gastrointestinal infection of SARS-CoV-2, *Gastroenterology* (2020), doi: <https://doi.org/10.1053/j.gastro.2020.02.055>.
5. Li, F., Li, W., Farzan, M., & Harrison, S. (2005). Structure of SARS coronavirus spike receptor-binding domain complexed with its receptor. *Nature* 437: 439-444. doi:10.1038/nature04231

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