

**SEROMATRIX**  
Quantitative  
Anti-SARS-CoV-2 (COVID-19)  
IgG ELISA Kit

Catalog Number SMGQ-2101

96 Tests

For research use only

[www.seromatrix.com](http://www.seromatrix.com)

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## Introduction

### Intended Use

Anti-SARS-CoV-2 (COVID-19) Human IgG Assay Kit is a Quantitative indirect enzyme-linked immunoassay (ELISA) for detection of human IgG antibodies against SARS-CoV-2 (COVID-19) in human serum and plasma samples.

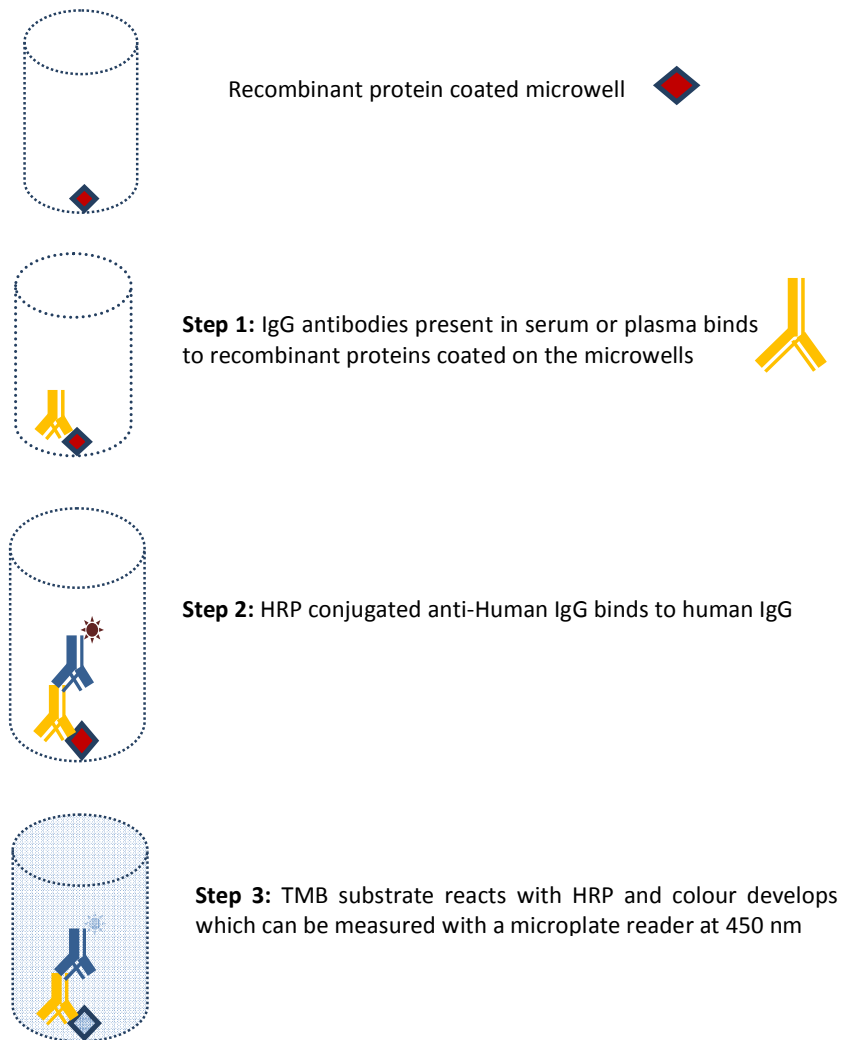
### Principle of the Assay

Quantitative determination of Anti-SARS-CoV-2 IgG antibodies is achieved by an indirect ELISA format, Microwells of the plate are pre-coated with recombinant proteins and stabilized using Seromatrix protein stabilizer.

(1°) The IgG antibodies to COVID-19 present in serum or plasma samples are captured following an incubation time of 30 minutes.

(2°) HRP labelled anti-human IgG antibodies bind the Fc region of the human IG antibodies present in the serum/plasma.

(3°) The TMB substrate reacts with HRP and develops a blue color depending on the presence of the IgG antibodies in the samples.



## General Information

### Materials Supplied

List of components

Component	Amount
<b>Positive control</b> Human Anti-SARS-CoV-2 IgG 1. Standard: 24 U/mL 2. Standard: 8 U/mL 3. Standard: 2.6 U/mL 4. Standard: 0.8 U/mL  <i>Ready to use</i> <b>Store at 4°C</b>	4x 0.5 ml
<b>Negative Control</b> <i>Ready to use</i> <b>Store at 4°C</b>	1 ml
<b>Sample Diluent</b> <i>Ready to use</i> <b>Store at 4°C</b>	100 ml
<b>96-Well Recombinant Protein Coated Microplate</b> <i>Ready to use</i> <b>Store at 4°C</b>	1 Plate
<b>Anti-human IgG antibody (HRP conjugated)</b> <i>Ready to use</i> <b>Store at 4°C</b>	10 ml
<b>Wash Buffer</b> <b>Store at 4°C</b>	250 ml
<b>TMB Reagent</b> <i>Ready to use</i> <b>Store at 4°C</b>	8 ml
<b>Stop Solution</b> <i>Ready to use</i> <b>Store at 4°C</b>	8 ml

### Storage Instruction

Upon receipt, the 96-Well COVID-19 Recombinant Protein Coated Microplate and TMB Reagent should be stored at 4°C.

Stop Solution should be stored at room temperature.

The Anti-human IgM antibody (HRP) and Anti-human IgG antibody (HRP) should be stored at +4°C.

### Materials Required but Not Supplied

The following materials and equipment are NOT provided in this kit but are necessary to successfully conduct the experiment:

- ✓ Microplate reader able to measure absorbance at 450 nm.
- ✓ Micropipettes with capability of measuring volumes ranging from 1 µL to 1 mL.
- ✓ Deionized or sterile water.
- ✓ Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer.
- ✓ Graph paper or computer software capable of generating or displaying logarithmic functions.
- ✓ Paper towels
- ✓ Test tubes or microfuge tubes capable of storing  $\geq 1$  mL.
- ✓ ELISA shaker.

## **Precautions for Use**

### **Assay Restrictions**

- ✓ This ELISA kit is intended for research purposes only
- ✓ Materials included in this kit should NOT be used past the expiration date on the kit label.
- ✓ Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- ✓ Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.

### **Health and safety precautions**

- ✓ Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
- ✓ Stop Solution contains H<sub>2</sub>SO<sub>4</sub> and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate or strips.

## **Assay Procedure**

### **Dilution of patient samples**

Dilute the patient samples 1:101 by adding **10 µL of patient sample** into **1 ml of Sample Diluent** in a 2 ml microcentrifuge tube or 2 ml tube.

Vortex well.



Diluted sample is stable up to 24 hr at +4°C.

1. Add 100 µL of each **standard** (ready to use) to 4 wells.
  - 1) Standard: 24 U/mL
  - 2) Standard: 8 U/mL
  - 3) Standard: 2.6 U/mL
  - 4) Standard: 0.8 U/mL
2. Add 100 µL of 1:101 diluted **Human Serum** or plasma samples into the corresponding wells of the microtiter plate.  
Incubate for 30 minutes at room temperature.
3. Wash 5 times with 250 µL of 1x Wash buffer.
4. Add 100 µL of Anti-human **IgG** antibody (HRP) Diluent to corresponding wells and incubate for 30 minutes at room temperature
5. Wash 5 times with 250 µL of 1x Wash buffer volume per well.
6. Add 50 µL of TMB Reagent to each well and incubate for 15 minutes at room temperature in the dark. TMB Reagent should be return to room temperature before use  
*Note: TMB Reagent is a light-sensitive reagent. Keep away from light.*
7. Add 50 µL of Stop Solution to each well and read OD at 450 nm immediately using the microplate reader.

### **Quality Control Value**

Before proceeding to calculate results, make sure that absorbance values obtained for the reagent blank and controls fall within the guidelines presented in Table 1.

**Table 1:** Quality control values

Sample	Expected Result
Reagent blank Absorbance	< 0.150
Negative control Absorbance	< 0.200
Positive control standards	See labeling of the each standard

The results are valid only if the absorbance value of blank does not exceed 0.100 and the mean absorbance value of negative control does not exceed 0.200

### **Results calculation:**

Subtract the blank from absorbance read outs of all patient samples and IgG standards

Calculate in excel or using a software the IgG concentrations of each sample against IgG level standards (see assigned value of each).

### **Interpretation of the results**

<b>Positive:</b> ≥5 U/mL
<b>Borderline:</b> ≥4U/mL-4.99 U/mL
<b>Negative:</b> <4 U/mL

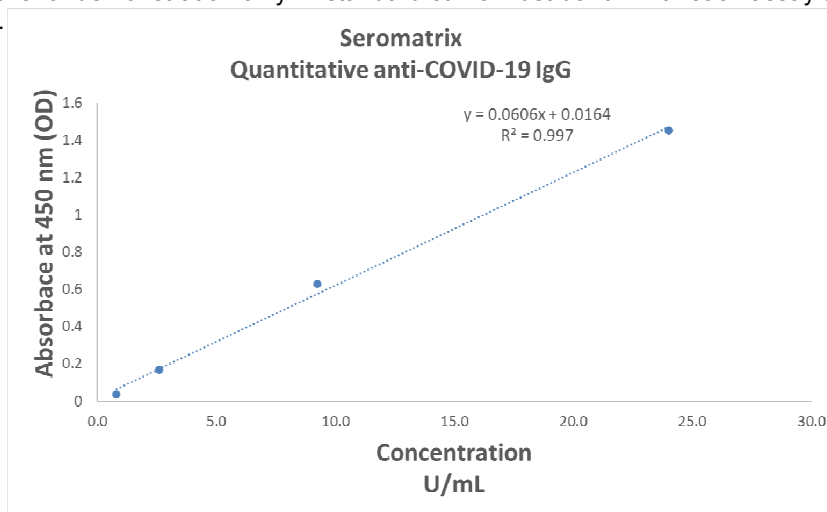
The manufacturer recommended P<sub>97.5</sub> positive cut-off level is **≥5 U/mL**.

It is highly recommended that each laboratory should establish their own cut-off level for COVID-19 IgG based on local population and disease prevalence.

### **Typical data of standard curve**

STANDARD	CONCENTRATION	ABSORBANCE (Blank reduced)
STANDART 1	24 U/mL	1.435
STANDART 2	8 U/mL	0.369
STANDART 3	2.6 U/mL	0.154
STANDART 4	0.8 U/mL	0.027
Negative Control	1.6 U/mL	0.111

This standard curve is for demonstration only. A standard curve must be run with each assay and prepared using excel or a software.



## Clinical Testing

Single serum samples of hospitalized 88 patients and 81 negative donors have been tested with **Seromatrix** COVID-19 Nucleocapsid IgG Quantitative ELISA on 26.01.2021.

The criteria for positive results were as follows:

### **Seromatrix** COVID-19 Nucleocapsid IgG Quantitative ELISA

- Positive:  $\geq 5$  U/mL
- Borderline:  $\geq 4$ U/mL-4.99 U/mL
- Negative:  $< 4$  U/mL

### **Results:**

18 Covid patients out of 88 found to be IgG positive.

<b>Statistic</b>	<b>Value</b>	<b>95% CI**</b>
Specificity	100%	95.55% to 100.00%
Positive Predictive Value (*)	100%	
Negative Predictive Value (*)	98%	98.23% to 98.56%
Accuracy (*)	98%	95.17% to 99.71%

(\*) These values are dependent on disease prevalence.

(\*\*) Confidence Interval

**Conclusion:** Covid-19 IgG development may take at least 15 days following the infection. IgG testing at early days of infection may yield false IgG results or has limited value as the level of IgG may not be detectable yet. Covid-19 IgG testing has limitations at early acute stage of infection and must be used with caution.

Asymptomatic patients should be followed weekly for at least 4 weeks.

## LIMITATIONS OF THE PROCEDURE

1. Since there is no Gold Standard concentration available for COVID-19 IgG measurement, the values of the assay calibrators were established by diluting COVID-19 IgG positive serum stock in a phosphate buffer protein matrix.
2. In the first week of the onset of the infection with the novel coronavirus (COVID-19), patient results may be negative for IgG. In addition, 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 light IgG positive result due to similarity of different strains.
3. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.
4. Results are for the detection of SARS CoV-2 antibodies. IgG Antibodies to SARS-CoV-2 are generally detectable in blood within 2 weeks after initial infection, although the duration of time antibodies are present post-infection is not well characterized.
5. The sensitivity of **Seromatrix** Anti-SARS-CoV-2 (COVID-19) IgG ELISA within 15 days of infection is unknown. Negative results do not rule out acute SARS-CoV-2 infection. If acute infection is suspected, PCR testing for SARS-CoV-2 is necessary.
6. False positive results for **Seromatrix** COVID-19 Nucleocapsid IgG Quantitative ELISA Kit may occur due to cross-reactivity from preexisting antibodies or other possible causes.
7. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, PCR testing for SARS-CoV-2 is necessary.
8. Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.
9. Positive results may also be due to past or present infection with nonSARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
10. For samples that do not align with PCR testing, perform a confirmation test by retesting the sample 2-3 times after 4 to 8 days.

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## **TECHNICAL ASSISTANCE AND CUSTOMER SERVICE**

For technical assistance or place an order, please contact Seromatrix Diagnostics, at (90) 532 66096 68 or by email: [hakansavli@yahoo.com](mailto:hakansavli@yahoo.com).

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