GOLD STANDARD℠
DIAGNOSTICS

# SARS-CoV-2

# IgG ELISA Test Kit

**FOR PRESCRIPTION USE ONLY**

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# Contents

## 1.0 Intended Use

The Gold Standard Diagnostics SARS-CoV-2 IgG ELISA Test Kit is intended for the qualitative detection of IgG antibodies to SARS-CoV-2 virus in human serum to aid in the diagnosis of Coronavirus (COVID-19).

## 2.0 Summary and Explanation

At the end of 2019, a novel respiratory disease emerged in Hubei Province of the People's Republic of China and soon spread to many other countries all over the world. The causative agent was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On March 11, 2020 the World Health Organization (WHO) declared the outbreak as a pandemic.

Coronaviruses are enveloped, positive single-stranded large RNA viruses that infect humans, but also a wide range of animals. The common human Coronaviruses NL63, 229E, OC43 and HKU1 are widespread especially throughout the winter months and are responsible for 5 to 30% of all acute respiratory diseases, typically with mild symptoms (common cold). More than 80% of the adult population have antibodies against human Coronaviruses. The immunity from previous infections last only for a short period of time and reinfections with the same pathogen is possible just after one year. Special interest was raised by two novel beta-coronaviruses that caused severe respiratory syndromes of the lower respiratory tract: SARS coronavirus that caused a worldwide epidemic in 2003 and MERS coronavirus that caused several hundred infections in 2012, especially in the middle east.

Four subfamilies, namely alpha-, beta-, gamma- and delta-coronaviruses exist. While alpha- and beta-coronaviruses apparently originate from mammals, in particular from bats, gamma- and delta-viruses originate from pigs, birds, and aquatic animals. The novel coronavirus SARS-CoV-2 belongs to the B lineage of the beta-coronaviruses and is closely related to the SARS-CoV virus. SARS-CoV-2 apparently succeeded in making its transition from animals to humans on the Huanan seafood market in Wuhan, China (1-4).

The Gold Standard Diagnostics Coronavirus SARS-CoV-2 IgG ELISA Test is intended for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum to aid in the diagnosis of Coronoavirus (COVID-19) and is to be used in conjunction with clinical findings.

## 3.0 Test Principle

The Gold Standard Diagnostics SARS-CoV-2 IgG ELISA test consists of antigenic proteins specific for SARS-CoV-2 which are transferred to polystyrene 96 well microtiter plates.

During the test procedure, antibodies to SARS-CoV-2, if present in the human serum sample will bind to the antigens coated onto the wells forming antigen-antibody complexes. Excess antibodies are removed by washing. A conjugate of goat anti-human IgG antibodies conjugated with horseradish peroxidase are then added, which binds to the antigen-antibody complexes. Excess conjugate is removed by washing. This is followed by the addition of a chromogenic substrate, tetramethylbenzidine (TMB). If specific antibodies to the antigen are present in the patients' serum, a blue color will develop. The enzymatic reaction is then stopped with a stopping solution

causing the contents of the well to turn yellow. The wells are read photometrically with a microplate reader at 450nm.

## 4.0 Package Contents

1. **96 well breakable microtiter plate,** coated with SARS-CoV-2 antigen, stabilized in a foil pouch, ready to use.
2. **Dilution Buffer, 2 x 50ml,** with preservative and Tween 20, ready to use.
3. **Wash Concentrate 20X, 50ml**, with preservative and Tween 20. Dilute with 1000ml distilled or deionized water before use.
4. **Negative Control, 1.5ml,** human serum with protein-stabilizer and preservative, ready to use.
5. **Calibrator, 1.5ml**, human serum with protein-stabilizer and preservative, ready to use.
6. **Positive Control, 1.5ml,** human serum with protein-stabilizer and preservative, ready to use.
7. **Conjugate IgG, 13.5ml**, goat anti-human IgG antibodies with protein stabilizer and preservative, ready to use.
8. **Substrate Solution (TMB), 13.5ml**, ready to use.
9. **Stop Solution, 7.5ml,** ready to use.

## 5.0 Storage and Stability

Store the test kit at 2-8°C. The shelf life of all components is shown on each respective label. The components are good for one month once opened provided it has not yet reached its expiration date.

1. Do not freeze kit components.
2. Do not use the kit reagents after their expiration date.
3. Do not expose reagents to strong light during storage or incubation.
4. The substrate solution (TMB) is light sensitive and must be stored in the dark.
5. Unused microtiter wells can be resealed in foil pouch with desiccant and stored at 2-8°C for up to one month.

## 6.0 Precautions and Warnings

1. For *in vitro* diagnostic use only.
2. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis B surface antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be handled as potentially infectious material.
3. The disposal of the used materials must be done according to the country-specific guidelines.

## 7.0 Material Required but not Supplied

1. Deionized or distilled water.
2. Eight channel pipette: 50ul, 100ul
3. Micropipettes: 10ul, 100ul, 1000ul
4. Test tubes
5. Paper towels or absorbent paper

6. Cover for ELISA plates
7. ELISA handwasher or automated ELISA plate washing device
8. Spectrophotometer with wavelength 450nm and 620nm

## 8.0 Test Procedure

Follow the instructions in the package insert to ensure correct results.

### 8.1 Samples

1. Dilute patient sera 1:101 with Dilution Buffer. Use fresh patient sera dilutions.
2. Serum samples for testing can be held at 2-8°C for at least 7 days and can withstand up to 3 freeze and thaw cycles. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.
3. Do not use heat-inactivated sera.
4. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not be used.

### 8.2 Preparation of Reagents

The ready to use controls (positive control, calibrator, and negative control) are kit lot specific and should only be used with the plate lot indicated in the Quality Control Certificate.

1. Bring all reagents to room temperature before starting the test.
2. Dilute Wash Concentrate with 1000ml distilled or deionized water before use. Mix well.
3. Shake/mix all liquid components well before use.

### 8.3 Test Procedure

1. Add 100ul of Controls, diluted Patient Samples, and Dilution Buffer (blank) into assigned wells.
2. Incubate for 30 minutes at room temperature.
3. Wash wells 3 times with 350-400ul of diluted wash buffer.
4. Remove residual wash solution by blotting on paper towels or absorbent pads.
5. Add 100ul of Conjugate per well.
6. Incubate for 30 minutes at room temperature.
7. Wash wells 3 times with 350-400ul of diluted wash buffer.
8. Remove residual wash solution by blotting on paper towels or absorbent pads.
9. Add 100ul of Substrate per well.
10. Incubate for 30 minutes at room temperature.
11. Add 50ul of Stop Solution per well.
12. Read wells on an ELISA plate reader (spectrophotometer) at 450nm with a reference wavelength of 620nm (620-690nm). Wells should be read within 30 minutes after adding the Stop Solution.

## 9.0 Quality Control

Results are valid only if the control values are in the acceptable range stated in the Quality Control Certificate. The positive control and negative control are intended to monitor for substantial reagent failure but will not ensure precision at the assay cut-off. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Refer to CLSI document C24 guidance on appropriate QC practices.

### 9.1 Test Function Control

1. The OD of the blank should be <0.150.
2. The OD of the Calibrator should be greater than that of the Negative Control.

### 9.2 Calculation of Units

1. The OD of the blank value should be subtracted from all other OD values.
2. The **Cutoff** is determined by multiplying the Calibrator OD (or mean OD) by the Correction Factor value printed on the Calibrator vial label and on the Quality Control Certificate.

**Cutoff = OD Calibrator x Correction Factor (C.F.)**

**Units* = (OD Controls or Samples / Cutoff) x 10**

*Units are qualitative. They are values defined by the manufacturer.

### 9.3 Interpretation of Results

| Units | Results | Interpretation |
|---|---|---|
| <9.0 | Negative | No detectable antibodies to SARS-CoV-2. 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. |
| 9.0 – 11.0 | Equivocal | Equivocal results should only be interpreted as initial evidence for detection of antibodies to SARS-CoV-2. Additional testing is recommended for equivocal test results. Serological evidence is best obtained by testing of paired acute and convalescent phase samples obtained several weeks apart. |
| >11.0 | Positive | Antibodies to SARS-CoV-2 detected. 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. |

## 10.0 Limitations

1. Test reports must include the following information:
   - This test has not been reviewed by the FDA.
   - The test is an aid in the diagnosis and is to be used in conjunction with clinical findings. The results should not be used as a sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
   - 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.
   - 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.
   - Not for the screening of donated blood.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera may cause erroneous results and should not be used.
3. The assay performance characteristics have not been established for matrices other than serum.
4. Some cross reactivity in patients with Respiratory Syncytial Virus was observed with the Gold Standard Diagnostics SARS-CoV-2 IgG ELISA Test Kit as shown in the Cross Reactivity Section. A dual infection with SARS-CoV-2 is not ruled out in these specimens, however, interpret results from these patients with caution.

## 11.0 Performance Characteristics

### Clinical Performance

### Diagnostic Sensitivity

The sensitivity was determined by testing samples positive for SARS-CoV-2 by RT-PCR on the SARS-CoV-2 IgG ELISA Test Kit. The following table show the results sorted by the timing post symptom onset:

| | | GSD SARS-CoV-2 IgG ELISA Test Kit | | | |
|---|---|---|---|---|---|
| **Days Post Symptom Onset** | **Number of Samples** | **Positive** | **Equivocal** | **Negative** | **Sensitivity** |
| Day 0-5 | 13 | 1 | 0 | 12 | 7.7% |
| Day 6-8 | 10 | 3 | 0 | 7 | 30.0% |
| Day 9-11 | 10 | 4 | 0 | 6 | 40.0% |
| Day ≥ 12 | 9 | 9 | 0 | 0 | 100.0% |

### Diagnostic Specificity

The specificity was determined by testing healthy blood donor samples which were collected prior to the COVID-19 outbreak (collected before 2019) on the Gold Standard Diagnostics SARS-CoV-2 IgG ELISA Test Kit. The results are summarized in the following table:

| | | GSD SARS-CoV-2 IgG ELISA Test Kit | |
|---|---|---|---|
| | Number of Samples | Number Positive/Equivocal | Specificity |
| **Blood Donors - USA** | 50 | 0 | 100.0% |
| **Blood Donors - Germany** | 75 | 0 | 100.0% |

## Class Specificity

Samples tested positive for IgG antibodies by the Gold Standard Diagnostics SARS-CoV-2 IgG ELISA Test were treated with RF-Adsorption solution to remove IgG class antibodies. Untreated and treated samples were tested in parallel to demonstrate the class specificity. The results are summarized in the following table:

| GSD SARS-CoV-2 IgG ELISA Test Kit | | | | |
|---|---|---|---|---|
| | Untreated | | Treated | |
| **Sample** | **Units** | **Result** | **Units** | **Result** |
| **1** | 25.7 | Positive | 0.0 | Negative |
| **2** | 38.9 | Positive | 0.1 | Negative |
| **3** | 33.1 | Positive | 0.4 | Negative |
| **4** | 37.6 | Positive | 0.6 | Negative |

## Cross Reactivity

A study using 110 samples was conducted to evaluate potential cross reactivity from different pathogen and disease conditions. The samples were confirmed for their positivity for each respective marker or clinical diagnosis using legally marketed devices. The samples were tested on the Gold Standard Diagnostics SARS-CoV-2 IgG ELISA Test. The results are summarized in the following table:

| Pathogen / Diagnosis | Number of Sera Tested | # Positive / (%) |
|---|---|---|
| Adenovirus | 10 | 0 / (0%) |
| Parainfluenza virus | 9 | 0 / (0%) |
| Influenza A | 10 | 0 / (0%) |
| Influenza B | 10 | 0 / (0%) |
| Enterovirus | 10 | 0 / (0%) |
| Respiratory syncytial virus | 10 | 1 / (10%) |
| *Chlamydia pneumoniae* | 10 | 0 / (0%) |
| *Haemophilus influenza* | 3 | 0 / (0%) |
| *Legionella pneumophila* | 8 | 0 / (0%) |
| *Bordetella pertussis* | 10 | 0 / (0%) |
| *Mycoplasma pneumoniae* | 10 | 0 / (0%) |
| *Candida albicans* | 10 | 0 / (0%) |

## 12.0 References


1. *Zhou et al. (2020) A pneumonia outbreak associated with a* new coronavirus of probable bat origin, Nature, 579: 270-273
2. *Woo et al. (2004) Detection of Specific Antibodies to Severe Acute Respiratory Syndrome (SARS) Coronavirus Nucleocapsid Protein for Serodiagnosis of SARS Coronavirus Pneumonia, Journal of Clinical Microbiology, 42:2306-2309*
3. *Xiao et al. (2020) Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring, Journal of Medical Virology, 92:464–467.*
4. *Okba et al. (2020) SARS-CoV-2 specific antibody responses in COVID-19 patients, medRxiv 2020.03.18.20038059; doi: https://doi.org/10.1101/2020.03.18.20038059*