# SARS-CoV-2 IgG Seroconversion ELISA Kit

Catalog # ICOV2IGGKT
Strip well format. Reagents for up to 96 tests.
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## INTENDED USE

This SARS-CoV-2 IgG seroconversion assay is intended for the qualitative detection of SARS-CoV-2 specific antibodies of isotype IgG in human serum.
**For research use only.**

This test has not been reviewed by the FDA. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 with a molecular diagnostic is necessary. Results from antibody testing should not be used as the sole basis to diagnose or exclude acute SARS-CoV-2 infection. 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.

## BACKGROUND

SARS-CoV-2 is the novel coronavirus that causes CoronaVirus Disease 2019 (COVID-19). Serological assays are critical for characterizing immune responses to viral infections by determining the presence of viral antigen specific antibodies in infected and recovered patient sera.

## ASSAY PRINCIPLE

SARS-CoV-2 specific antibodies will bind to the purified recombinant HEK cell derived receptor-binding domain (RBD) of the SARS-CoV-2 spike protein coated on the microtiter plate. After appropriate washing steps, horseradish peroxidase labeled polyclonal anti-human IgG antibody binds to the captured protein. Excess antibody is washed away and TMB substrate is used for color development at 450 nm. Samples that exceed a determined cut-off value are designated positive by this assay.

## REAGENTS PROVIDED

- **96-well antigen coated microtiter strip plate** (removable wells 8x12) containing purified recombinant SARS-CoV-2 antigen, blocked and dried
- **10X Wash buffer:** 1 bottle of 50 ml
- **Sample buffer:** 1 bottle of 40 ml
- **Positive control:** 1 vial lyophilized
- **Negative control:** 1 vial lyophilized
- **Antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10 ml
- **Stop solution:** 1 bottle of 5 ml

## STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconsitituted controls and antibody may be frozen for later use. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

## OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Tubes for dilution of samples
- Paper towels or laboratory wipes


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## PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.
- Controls of human origin have tested negative for common pathogens. However all reagents should be treated as being a potential infection hazardand should be handled with care.
- As a general safety precaution the assay may be performed in a biological safety cabinet if desired.

## PREPARATION OF REAGENTS

- **1X Wash buffer:** Dilute 50 ml of 10X wash buffer concentrate with 450 ml of deionized water.

## PREPARATION OF SAMPLES

Perform peripheral blood draw by venipuncture into a red top or serum separator tube. Invert, clot, and centrifuge tube according to manufacturer directions. Patient samples are diluted 1:51 in sample buffer. For example, add 5 µl to 250 µl of sample buffer and mix well by vortexing.

## PREPARATION OF CONTROLS

Briefly centrifuge vials before opening. Reconstitute positive and negative controls by adding 500 µl of sample buffer directly to each vial and agitating gently to completely dissolve contents.

## ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300 rpm) at each step of the assay.

### Sample Addition

Remove microtiter plate from bag and add 100 µl of controls and diluted samples to wells in duplicate according to the suggested plate layout. Carefully record position of samples. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

### Antibody Addition

Briefly centrifuge vial before opening. **See C of A for lot specific dilution instructions.** Add 100µl of your dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Substrate Incubation

Add 100 µl TMB substrate to all wells and shake plate for 5 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 µl of stop solution to all wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

### Measurement

Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. Subtract blank from all samples to determine corrected absorbance ($A_{450}$).

### Interpretation of Results

The following cut-off $A_{450}$ values are suggested for designating samples as positive or negative by this assay. The average of duplicate samples should be taken. If duplicate values differ substantially from one another it is suggested to retest the samples on the same assay. If samples fall in the indeterminate range it is suggested to either retest on the same assay, retest on an alternative assay, or to retest in 1-2 weeks. If retested in 1-2 weeks and the result remains indeterminate, the results should be discussed with the ordering physician.

| $A_{450}$ | Result |
|---|---|
| < 0.3 | Negative |
| ≥ 0.3 to < 0.5 | Indeterminate |
| ≥ 0.5 | Positive |



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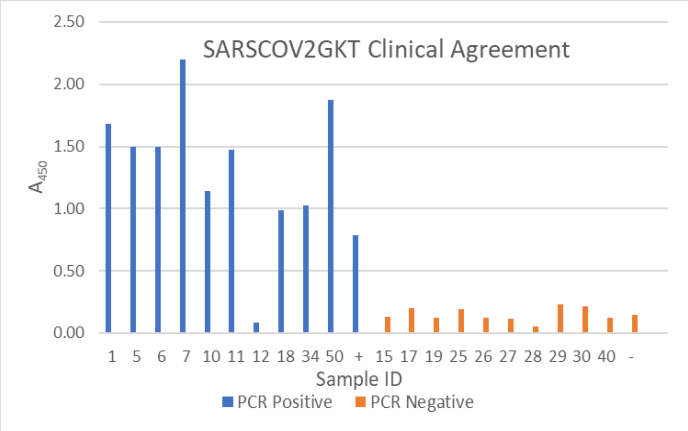
## EXPECTED VALUES

Significant seroconversion as demonstrated by antibodies specific to both the full length SARS-CoV-2 spike protein and the RBD region has been detected as early as two days post symptom onset [1].

## PERFORMANCE CHARACTERISTICS

**Variability:** A total of 136 samples were evaluated in duplicate yielding a median CV of 4.2% (95% CI 2.9-5.2%).

**Clinical Agreement:** Human serum samples from patients with PCR confirmed COVID-19 infection status were evaluated in the assay.




| | | PCR | |
|---|---|---|---|
| SARSCOV2GKT | | Positive | Negative |
| | Positive | 9 | 0 |
| | Negative | 1 | 10 |

**Sensitivity:** 90
**Specificity:** 100
**Positive Percent Agreement:** 100
**Negative Percent Agreement:** 91

**Assay Performance:** Human serum samples from patients with PCR confirmed COVID-19 infection status were collected at various time points following symptom onset were evaluated in the assay along with control samples collected prior to March 2020.

| COVID-19 PCR-Positive Samples | | | |
|---|---|---|---|
| Days Since Symptoms Onset | n | Sensitivity[1] | Sensitivity[2] |
| <15 days | 9 | 22.2% | 25.0% |
| >14 days | 37 | 91.9% | 97.1% |
| >21 days | 32 | 96.9% | 100.0% |
| Control Samples | | | |
| | n | Specificity[1] | Specificity[2] |
| | 89 | 100.0% | 100.0% |

[1]Calculated with indeterminate results classified as negative
[2]Calculated with indeterminate results excluded

**Cross Reactivity:** Human serum samples collected between April-June 2019 and pathogen positive samples were evaluated in the assay.

| Condition | n | Specificity[1] | Specificity[2] |
|---|---|---|---|
| Pre-COVID-19 | 101 | 98.0% | 98.0% |
| ENA/ANA | 15 | 100.0% | 100.0% |
| Anti-dsDNA positive | 5 | 100.0% | 100.0% |
| Hepatitis B and C | 10 | 100.0% | 100.0% |
| CoV 229E | 2 | 100.0% | 100.0% |
| CoV HKU1 | 8 | 100.0% | 100.0% |
| CoV OC43 | 2 | 100.0% | 100.0% |
| Non-specified seasonal CoV | 16 | 100.0% | 100.0% |

[1]Calculated with indeterminate results classified as negative
[2]Calculated with indeterminate results excluded

**Class Specificity:** Human serum samples with positive serology were treated with dithiothreitol (DTT) and the following results were observed, indicating IgG class specificity.

| Sample | Result No DTT (IgM/IgG) | Result DTT (IgM/IgG) | Expected Result DTT (IgM/IgG) | Result Agreement |
|---|---|---|---|---|
| 1 | +/+ | -/+ | -/+ | Yes |
| 2 | +/+ | -/+ | -/+ | Yes |
| 3 | +/+ | -/+ | -/+ | Yes |
| 4 | +/+ | -/+ | -/+ | Yes |
| 5 | +/+ | -/+ | -/+ | Yes |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## DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and should be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product. This test should not be used as the sole basis for treatment or other patient management decisions. Health care personnel should consider other information, including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an adaptive immune response.

## REFERENCES


1. Amanat F, *et al.*: medRxiv 2020.03.17.20037713; doi: https://doi.org/10.1101/2020.03.17.20037713


## MANUFACTURER CONTACT INFORMATION


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**Example of Suggested ELISA Plate Layout**
**96 Well Plate: 6 Control wells, 90 Sample wells**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | Blank | Negative Control | Positive Control | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 |
| **B** | Blank | Negative Control | Positive Control | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 |
| **C** | Sample 10 | Sample 11 | Sample 12 | Sample 13 | Sample 14 | Sample 15 | Sample 16 | Sample 17 | Sample 18 | Sample 19 | Sample 20 | Sample 21 |
| **D** | Sample 10 | Sample 11 | Sample 12 | Sample 13 | Sample 14 | Sample 15 | Sample 16 | Sample 17 | Sample 18 | Sample 19 | Sample 20 | Sample 21 |
| **E** | Sample 22 | Sample 23 | Sample 24 | Sample 25 | Sample 26 | Sample 27 | Sample 28 | Sample 29 | Sample 30 | Sample 31 | Sample 32 | Sample 33 |
| **F** | Sample 22 | Sample 23 | Sample 24 | Sample 25 | Sample 26 | Sample 27 | Sample 28 | Sample 29 | Sample 30 | Sample 31 | Sample 32 | Sample 33 |
| **G** | Sample 34 | Sample 35 | Sample 36 | Sample 37 | Sample 38 | Sample 39 | Sample 40 | Sample 41 | Sample 42 | Sample 43 | Sample 44 | Sample 45 |
| **H** | Sample 34 | Sample 35 | Sample 36 | Sample 37 | Sample 38 | Sample 39 | Sample 40 | Sample 41 | Sample 42 | Sample 43 | Sample 44 | Sample 45 |



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