Version 01-06/20

User's Manual

SARS-CoV-2 IgG ELISA Kit

REF **DEIASL019**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

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. This kit is provided for professional use only by clinical laboratories certified to perform moderate or high complexity tests.

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.

General Description

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.

Principles of Testing

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 secondary antibody binds to the captured protein. Excess secondary 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 And Materials Provided

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

Materials Required But Not Supplied

1. Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
2. Manifold dispenser/aspirator or automated microplate washer

3. Microplate reader capable of measuring absorbance at 450 nm
4. Pipettes and Pipette tips
5. Deionized or distilled water
6. Tubes for dilution of samples
7. Paper towels or laboratory wipes

Storage

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconsitituted controls and secondary 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.

Specimen Collection And Preparation

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.

Plate Preparation

96 Well Plate: 6 Standard wells, 90 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Negative	Positive Control	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
B	Blank	Negative	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

Reagent Preparation

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

Preparation of Control: 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.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Reconstitute HRP conjugated anti-human IgG by adding 500 µl of sample buffer directly to the vial and agitating gently to completely dissolve contents. Add 200 µl to 10 ml sample buffer and mix well. Add 100 µl to all wells. 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.

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

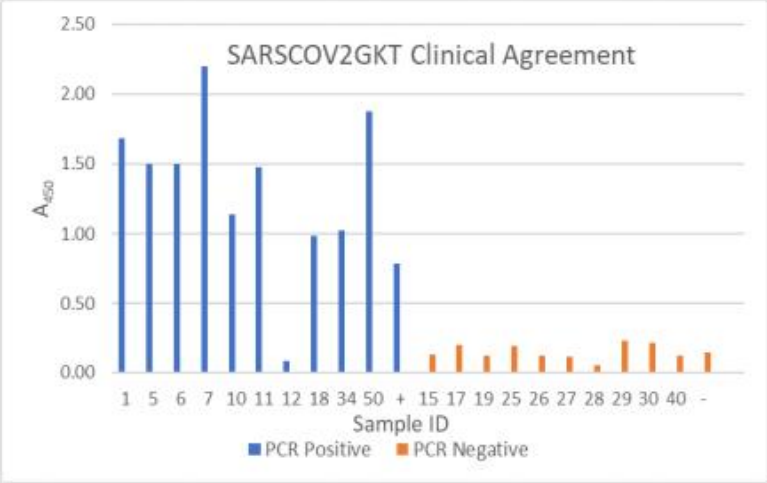
Reference Values

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.

Precision

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.



Sensitivity

		PCR	
SARSCOV2GKT		Positive	Negative
	Positive	9	0
	Negative	1	10

Sensitivity: 90

Specificity: 100

Positive Percent Agreement: 100

Negative Percent Agreement: 91

Specificity

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

Precautions

1. FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
2. 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.
3. Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
4. Keep plate covered except when adding reagents, washing, or reading.
5. DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
6. DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.
7. 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.
8. As a general safety precaution the assay may be performed in a biological safety cabinet if desired.