COVID-SeroIndex

Kantaro Quantitative SARS-CoV-2 IgG Antibody IVD Kit

Powered by R&D Systems®

REF DSR200-CE

For quantitative detection of human IgG antibodies to the SARS-CoV-2 virus in serum and plasma (K_2-EDTA/Li-Heparin) samples.

This kit contains sufficient materials to test 360 samples provided the assay is performed as described in this document.

If there is any evidence in damage to the external or internal packaging, do not use this kit.
Contact Bio-Techne Customer Care at 1-800-343-7475 or customerservice.na@bio-techne.com.

This package insert must be read in its entirety before using this product.
For *in vitro* Diagnostic use.

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DESCRIPTION & INTENDED USE

The COVID-SeroIndex, Kantaro Quantitative SARS-CoV-2 IgG Antibody IVD Kit consists of two serial direct Enzyme-Linked Immunosorbent Assays (ELISA) intended for quantitative detection of human IgG antibodies to the SARS-CoV-2 virus in serum and plasma (Li-Heparin and K_2-EDTA) specimens collected from individuals suspected by their healthcare provider of prior infection with the SARS-CoV-2 virus that causes COVID-19.

An initial qualitative ELISA is performed against recombinant Receptor Binding Domain of SARS-CoV-2, followed for positive specimens by a quantitative ELISA against full length SARS-CoV-2 Spike protein. The assay aids in establishing the quantitative levels of neutralizing antibodies indicative of an adaptive immune response to SARS-CoV-2 in patients suspected of previous SARS-CoV-2 infection, or for the detection of IgG seroconversion in patients following known recent SARS-CoV-2 infection.

Determination of the number of individuals who are demonstrated to have developed specific antibodies to SARS-CoV-2 aids in the determination of seroprevalence in any geographic region or group of exposed individuals and may be indicative of the potential risk of reinfection. The results of the assay correlate with the neutralization of SARS-CoV-2 virus *in vitro*.

Results from the COVID-SeroIndex, Kantaro Quantitative SARS-CoV-2 IgG Antibody IVD Kit should not be used as the sole basis for diagnosis and should not be used for the diagnosis of patients with acute COVID-19 infection.

Results are for the detection of SARS-CoV-2 IgG antibodies. IgG antibodies to SARS-CoV-2 generally become detectable beginning 10-14 days following infection but may occur later. The presence of IgG antibodies, following previously negative testing, defines IgG antibody seroconversion following SARS-CoV-2 infection.

Negative results do not preclude acute SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. IgG antibodies may not be present for more than two weeks following infection, and patients may remain infectious during acute infection even if IgG antibody is present. Results must be combined with clinical observations, patient history, and epidemiological information. The sensitivity of the COVID-SeroIndex, Kantaro Quantitative SARS-CoV-2 IgG Antibody IVD Kit early after infection is unknown.

False positive results for IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Prevalence of SARS-CoV-2 infection in the area where testing has occurred should be considered when interpreting positive test results.

At this time, it is unknown how long SARS-CoV-2 IgG antibodies may persist following infection.

LIMITATIONS OF THE PROCEDURE

IVD

- FOR *IN VITRO* DIAGNOSTIC USE ONLY.
- For professional use only by appropriately trained personnel in compliance with ISO 15189, CLSI, or other applicable regional or facility compliance requirements.
- Samples must be pipetted within 15 minutes.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.
- The following were not evaluated for interference in this assay:
    a. Samples from pregnant women, especially multipara (women who had more than one pregnancy).
    b. Samples from patients previously infected with the closely related virus strains SARS-CoV and MERS-CoV.
    c. Samples from individuals treated with relevant medicines such as:
        - Antiviral drugs
        - Antibacterial drugs
        - Acetylsalicylic acid
        - Paracetamol
        - Ibuprofen
        - Anti-hypertensive drugs
        - Anti-diabetic drugs
        - Hydroxychloroquine

PRINCIPLE OF ASSAY

The 2-phase assay is an antigen-down enzyme immunoassay which utilizes a recombinant SARS-CoV-2 Spike protein RBD antigen pre-coated onto a 96-well microplate in phase 1. When the sample is added, antibodies found in the sample that recognize SARS-CoV-2 RBD antigen bind to the antigen coated plate and are retained in the well. After washing away unbound substances, an enzyme-linked monoclonal antibody specific for human IgG is added to the wells. Following a wash to remove any unbound enzyme-linked antibody, a substrate is added to the wells and color develops in proportion to the amount of IgG antibodies in the sample bound to the SARS-CoV-2 RBD antigen. The color development is stopped, and the intensity of the color is measured. Samples that have a measured value above a pre-determined cutoff are determined to be positive and tested in the 2nd phase ELISA.

Positive samples from phase 1 are evaluated on a second orthogonal ELISA to quantify the levels of IgG antibodies to the SARS-CoV-2 Spike protein. For this assay, a recombinant SARS-CoV-2 Spike protein is pre-coated onto a 96-well microplate and used to bind antibodies found in the sample. When the sample is added, antibodies found in the sample that recognize SARS-CoV-2 Spike protein bind the antigen coated plate and are retained in the well. After washing away unbound substances, an enzyme-linked monoclonal antibody specific for human IgG is added to the wells. Following a wash to remove any unbound enzyme-linked antibody, a substrate is added to the wells and color develops in proportion to the amount of IgG antibodies in the sample bound to the SARS-CoV-2 Spike protein. The color development is stopped, and the intensity of the color is measured. The signal from unknown samples is compared to a calibration curve to generate a final result in arbitrary units per milliliter (AU/mL).

TECHNICAL GUIDANCE

- **In order to achieve optimal performance, do not allow the pipette tip to touch the inside of the well while loading calibrators, controls, samples, or blanks.**
- Sample Buffer (part # 896968) may contain a precipitate. Mix well prior to use.
- When working with protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each calibrator, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	QUANTITY	DESCRIPTION	STORAGE OF OPENED MATERIAL
RBD Antigen Microplate	899281	4 plates	96 well polystyrene microplate coated with recombinant SARS-CoV-2 Spike protein RBD antigen, IVD.	Use a new plate for each assay. Discard after use.
Spike Protein Microplate	899282	5 plates	96 well polystyrene microplate coated with full length recombinant SARS-CoV-2 Spike protein, IVD.	
RBD Conjugate Concentrate - IgG ELISA	899283	1 vial	125 µL of 1000-fold concentrated monoclonal antibody specific to human IgG conjugated to horseradish peroxidase, IVD.	May be stored for up to 1 month at 2-8 °C.* Discard diluted solutions after use. 
Spike Conjugate Concentrate - IgG ELISA	899284	1 vial	125 µL of 1000-fold concentrated monoclonal antibody specific to human IgG conjugated to horseradish peroxidase, IVD.	
Conjugate Buffer - IgG ELISA	896967	1 bottle	120 mL of a buffered protein base with preservatives, IVD.	
Sample Buffer - IgG ELISA	896968	3 bottles	91 mL of a buffered protein base with preservatives, IVD. *May contain a precipitate. Mix well prior to use.*	
TMB Substrate - IgG ELISA	895276	1 bottle	116 mL of stabilized hydrogen peroxide and chromogen (tetramethylbenzidine), IVD.	
Stop Solution - IgG ELISA	895277	1 bottle	116 mL of acidic solution, IVD.	
Wash Buffer - IgG ELISA	895278	2 bottles	101 mL of a 25-fold concentrated solution of buffered surfactant with preservative, IVD.	

* Provided this is within the expiration date of the kit.

MATERIALS PROVIDED & STORAGE CONDITIONS *CONTINUED*

PART	PART #	QUANTITY	DESCRIPTION	STORAGE OF OPENED MATERIAL
RBD Positive Control	83700	1 vial	1.0 mL of monoclonal antibody in a protein buffered base with preservatives, IVD.	Store at 2-8 °C. Refer to vial label for expiration date.* Discard diluted solutions after use. 
RBD Negative Control	83701	1 vial	1.0 mL of a buffered protein base with preservatives, IVD.	
Spike Low Control	83702	1 vial	1.0 mL of monoclonal antibody in a protein buffered base with preservatives, IVD.	
Spike Mid Control	83703	1 vial	1.0 mL of monoclonal antibody in a protein buffered base with preservatives, IVD.	
Spike High Control	83704	1 vial	1.0 mL of monoclonal antibody in a protein buffered base with preservatives, IVD.	
Spike Calibrator 1 (0 AU/mL)	83705	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives, IVD.	
Spike Calibrator 2 (0.82 AU/mL)	83706	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives, IVD.	
Spike Calibrator 3 (2.47 AU/mL)	83707	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives, IVD.	
Spike Calibrator 4 (7.41 AU/mL)	83708	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives, IVD.	
Spike Calibrator 5 (22.2 AU/mL)	83709	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives, IVD.	
Spike Calibrator 6 (66.7 AU/mL)	83710	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives, IVD.	
Spike Calibrator 7 (200 AU/mL)	83711	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives , IVD.	

OTHER SUPPLIES REQUIRED

- Heat block or water bath
- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 25 mL and 500 mL graduated cylinders
- **Polypropylene** test tubes for dilution of samples
- Plate sealers (R&D Systems®, Catalog # DY992) (optional)

WARNINGS & PRECAUTIONS

- Some components in this kit contain human source materials and have been tested negative for antibodies to HIV 1&2, Hepatitis C and Hepatitis B surface antigen. Because no test method can offer complete assurance that infectious agents are absent, material should be handled as potentially infectious, following precautions as specified in the OSHA Bloodborne Pathogen Rule (29 CFR Part 1910, 1030) or other equivalent biosafety procedures.
- The Stop Solution provided with this kit is an acid solution.
- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Substrate may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.
- All waste should be disposed of in accordance to local regulations.

TABLE OF SYMBOLS

SYMBOL	MEANING
	Manufacturer's Catalog designation or number
	Use by, expiration date
	Lot Number
	CE mark in accordance to the European Medical Device Directive
	European Union Authorized Representative
	*In vitro* diagnostic medical device
	Consult instructions for use
	Caution or warning
	Health hazards
	Manufactured By
	Biological risks
	Corrosive
	Keep away from sunlight
	Keep dry
	Do not use if package is damaged and the product inside appears physically damaged.
	Temperature limits (example limits shown)
	Unique Device Identifier
	Package contents
	Prescription use only Caution: Federal law (USA) restricts this device to sale by or on the order of a licensed healthcare practitioner
	Intended Use

SAMPLE COLLECTION & STORAGE

If freezing samples, avoid repeat freeze-thaw cycles. Do not freeze samples more than 3 times.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at 4 °C for up to 7 days.

Plasma - Collect plasma using K_2-EDTA or Li-Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at 4 °C for up to 7 days.

Note: *Citrate plasma has not been validated for use in this assay.*

REAGENT PREPARATION

1X RBD Conjugate - For each plate, add 11 µL of RBD Conjugate Concentrate (1000-fold) (part #899283) to 11 mL of Conjugate Buffer (part # 896967). Mix well.

1X Spike Conjugate - For each plate, add 11 µL of Spike Conjugate Concentrate (1000-fold) (part #899284) to 11 mL of Conjugate Buffer (part # 896967). Mix well.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. For one plate, add 20 mL of Wash Buffer Concentrate (part # 895278) to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Control Preparation - Just prior to use, dilute each control 5-fold by pipetting 0.4 mL of Sample Buffer (part # 896968) into a tube. Add 0.1 mL of the control. Repeat for all 5 controls (RBD Positive, RBD Negative, Spike Low, Spike Mid, and Spike High). Make fresh for each plate.

Calibrators - No preparation required; calibrators are supplied ready to use.

SAMPLE PREPARATION

Note: *Samples must be heat inactivated prior to use in this assay.*

Heat Inactivation:

1. Heat inactivate samples by placing in a water bath or heat block at 56 °C for 1 hour.
   Note: *Do not leave samples at 56 °C for longer than 1 hour.*
2. Aliquot and store samples at 4 °C for up to 7 days post collection.

RBD Assay:

1. Dilute heat inactivated samples 5-fold in microcentrifuge tubes by adding 10 µL of sample to 40 µL of Sample Buffer.
2. Further dilute samples 20-fold (final 100-fold dilution) by adding 10 µL of diluted sample from step 1 (diluted 5-fold) to 190 µL of Sample Buffer.

Spike Assay:

1. Dilute heat inactivated samples 5-fold in microcentrifuge tubes by adding 10 µL of sample to 40 µL of Sample Buffer.
2. Further dilute samples 40-fold (final 200-fold dilution) by adding 10 µL of diluted sample from step 1 (diluted 5-fold) to 390 µL of Sample Buffer.

RBD ELISA ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
B	Pos Control	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
C	Neg Control	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
D	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
E	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
F	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	Blank
G	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	Pos Control
H	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	Neg Control

1. Add 100 µL of control (diluted 5-fold), heat inactivated sample (diluted 100-fold, test in singlets), or sample buffer (blank) per well. Incubate for 2 hours at room temperature on benchtop. Cover with an adhesive strip if needed.
   Note: *Pipette controls and samples within 15 minutes.*

2. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

3. Add 100 µL of 1X RBD Conjugate to each well. Incubate for one hour at room temperature. Cover with an adhesive strip if needed.

4. Repeat the aspiration/wash as in step 2.

5. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Protect from light.

6. Add 100 µL of Stop Solution to each well. The color in the well should change from blue to yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Determine the optical density of each well within 30 minutes (minimum 0 minutes, maximum 30 minutes), using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

RBD ELISA ASSAY PROCEDURE *CONTINUED*

RBD ELISA Calculation of Results:

The RBD Positive Control (diluted 5-fold), part # 83700, is used for normalization. Corrected sample OD values (see RBD ELISA step 7) are divided by the corrected RBD Positive Control (diluted 5-fold) OD value to calculate a cutoff index (CI) value.

$$\frac{\text{Corrected OD of the sample}}{\text{Mean of Corrected OD of the RBD Positive Control}} = \text{Cutoff Index (CI)}$$

If the calculated CI value is ≥ 0.70, the sample is considered RBD positive and requires confirmation using the Spike ELISA. If the CI value is < 0.7, the sample is negative and contained no detectable levels of antibodies to the RBD protein fragment of SARS-CoV-2 Spike protein.

RBD Quality Control:

Each testing laboratory should establish a quality control program to monitor the performance of the COVID-SeroIndex Immunoassay. As part of this program, controls with known anti-SARS-CoV-2 IgG concentrations (provided) should be tested in each assay. Satisfactory performance is obtained when controls fall within the established ranges provided in the Certificate of Analysis or within your interval, as determined by an appropriate internal laboratory quality control procedure. Follow your laboratory's quality control procedures; if the results obtained do not fall within the acceptable limits, the assay results may be invalid.

The corrected OD of the blank should be < 0.03 OD.

SPIKE ELISA ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal 1	Cal 1	S3	S11	S19	S27	S35	S43	S51	S59	S67	S72
B	Cal 2	Cal 2	S4	S12	S20	S28	S36	S44	S52	S60	S68	S73
C	Cal 3	Cal 3	S5	S13	S21	S29	S37	S45	S53	S61	S69	S74
D	Cal 4	Cal 4	S6	S14	S22	S30	S38	S46	S54	S62	S70	S75
E	Cal 5	Cal 5	S7	S15	S23	S31	S39	S47	S55	S63	S71	S76
F	Cal 6	Cal 6	S8	S16	S24	S32	S40	S48	S56	S64	Low	Low
G	Cal 7	Cal 7	S9	S17	S25	S33	S41	S49	S57	S65	Mid	Mid
H	S1	S2	S10	S18	S26	S34	S42	S50	S58	S66	High	High

1. Add 100 µL of control (diluted 5-fold), calibrator (undiluted), or RBD positive heat inactivated sample (diluted 200-fold, test in singlets) per well. Incubate for 2 hours at room temperature on benchtop. Cover with an adhesive strip if needed.
   Note: *Pipette calibrators, controls, and samples within 15 minutes.*

2. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

3. Add 100 µL of 1X Spike Conjugate to each well. Incubate for one hour at room temperature. Cover with an adhesive strip if needed.

4. Repeat the aspiration/wash as in step 2.

5. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Protect from light.

6. Add 100 µL of Stop Solution to each well. The color in the well should change from blue to yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Determine the optical density of each well within 30 minutes (minimum 0 minutes, maximum 30 minutes), using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

SPIKE ELISA ASSAY PROCEDURE *CONTINUED*

Spike ELISA Calculation of Results:
Read the absorbance of each well on a microplate reader using 450 nm as the primary wavelength and 540 nm or 570 nm as the reference wavelength. Average the duplicate readings for each calibrator and control.

Create a standard curve by reducing the calibrator values using computer software capable of generating a four-parameter logistic (4-PL) curve fit.

Samples falling below the Limit of Quantification (LoQ) of 3.20 AU/mL are considered negative. Values above the analytical measuring range should be reported as >160 AU/mL.

Spike Quality Control:
Each testing laboratory should establish a quality control program to monitor the performance of the COVID-SeroIndex Immunoassay. As part of this program, controls with known anti-SARS-CoV-2 IgG concentrations (provided) should be tested in each assay. Satisfactory performance is obtained when controls fall within the established ranges provided in the Certificate of Analysis or within your interval, as determined by an appropriate internal laboratory quality control procedure. Follow your laboratory's quality control procedures; if the results obtained do not fall within the acceptable limits, the assay results may be invalid.

INTERPRETATION OF RESULTS

Assessment of the COVID-SeroIndex Kit results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

NEGATIVE RBD Screen Result: Indicates that a 100-fold dilution of the sample tested contained no detectable levels of specific antibodies to the RBD protein fragment of SARS-CoV-2 Spike protein and no evidence of a detectable level of immune response to SARS-CoV-2 virus. The patient from whom the sample was obtained is presumed not to have been infected with SARS-CoV-2 virus at the time the sample was obtained. A negative result does not preclude the possibility of a very early immune response, which is not yet producing detectable levels of IgG antibodies specific to the antigen.

PRESUMPTIVE POSITIVE RBD Screen Result: The 100-fold dilution of the sample produced a positive reaction to the RBD protein fragment of SARS-CoV-2 Spike protein. This must be confirmed by testing its reactivity against the full-length Spike protein of the virus to confirm an appropriate level of circulating antibody in the tested sample.

ANTIBODY Concentration: The Quantitative ELISA Assay can reproducibly measure levels of SARS-CoV-2 IgG antibodies and the results are expressed in arbitrary units per milliliter of test sample. These numerical values have been experimentally shown to correlate with viral neutralizing activity *in vitro*. The experimentally determined measurable range is 3.2-160 AU/mL.

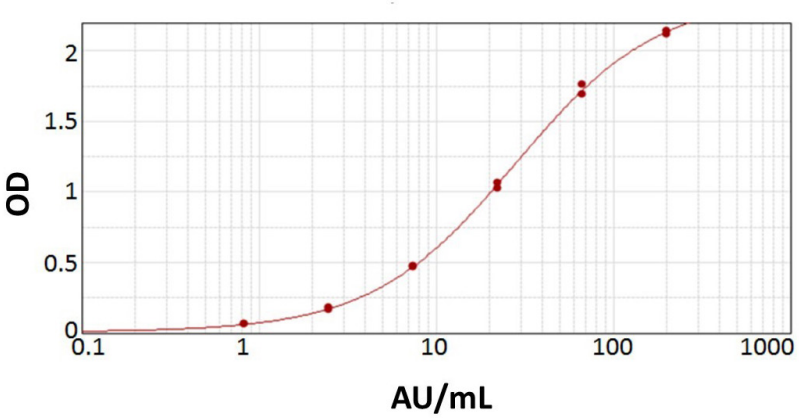
The circulating levels of IgG antibodies specific to the Spike protein of SARS-CoV-2 virus in AU/mL can be separated into clinically relevant levels correlated with SARS-CoV-2 neutralizing activity *in vitro*.

The use of AU/mL is an accepted method of quantitation in the absence of a traceable standard for exact measurement of the substance of analytical interest. These units are related in a direct proportional manner used to show the ratio of amount of analyte to a predetermined reference material.

ANTIBODY CONCENTRATION (AU/mL)	CLINICAL INTERPRETATION
< 3.2 AU/mL (LoQ)	Negative. No or very early immune response. Indeterminate immune status against SARS-CoV-2. If clinically indicated, repeat in 3 weeks.
3.2-10 AU/mL	Low positive. Likely very early immune response. If clinically indicated, repeat in 3 weeks.
10-25 AU/mL	Moderate antibody level. It has been demonstrated that greater than 90% of persons with this concentration of IgG level specific for SARS-CoV-2 exhibit viral neutralizing activity *in vitro*.
>25 AU/mL	High antibody level. Indicates a significantly elevated serum concentration, potentially a strong immune status. It has been demonstrated that 90-100% of persons with this concentration of IgG level specific for SARS-CoV-2 exhibit viral neutralizing activity *in vitro*.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each Spike plate.



Calibrator	AU/mL	Average OD
1	0	0.003
2	0.82	0.062
3	2.47	0.175
4	7.41	0.471
5	22.2	1.048
6	66.7	1.726
7	200	2.132

ANALYTICAL MEASURING RANGE

The RBD ELISA is a qualitative ELISA and there is no defined analytical measuring range (AMR). The output of the device is given in CI values. CI values are calculated by dividing the corrected OD value for unknown samples by the corrected OD value for the mean of the RBD Positive Control. Unknown samples with a CI ≥ 0.70 are considered positive on the RBD ELISA and unknown samples with a CI < 0.70 are considered negative on the RBD ELISA. Unknown samples that test positive on the RBD ELISA are then tested on the Spike ELISA while unknown samples that test negative on the RBD ELISA are given a final determination of negative.

The AMR for the Spike protein ELISA was determined through the results of the analytical validation studies as described below. This range is based on the LoQ for the lower limit of the measuring interval, the determination of the linear range as described in the linearity study, and the high calibrator, which is set at 200 AU/mL. The studies described below demonstrate precision and linearity across the AMR. Results of the quantitative Spike ELISA are reported in AU/mL. The claimed AMR is 3.2-160 AU/mL.

WITHIN-SITE IMPRECISION

RBD ELISA - Within-site repeatability was determined by measuring four serum samples in two tests per day, three replicates per test for three days. Positive and negative controls were also measured in two replicates per test, two tests per day for three days.

Sample	n	Mean (CI)	Repeatability		Total Within-Laboratory Precision	
			SD	%CV	SD	%CV
Negative Control	12	0.040	0.004	11.0	0.005	12.3
Positive Control	12	1.00	0.035	3.5	0.035	3.5
Sample 1	18	0.153	0.005	3.0	0.012	7.9
Sample 2	18	0.756	0.031	4.1	0.077	10.2
Sample 3	18	1.06	0.073	6.9	0.093	8.7
Sample 4	18	1.88	0.062	3.3	0.116	6.2

Spike ELISA - Within-site repeatability was determined by measuring three serum samples in two tests per day, three replicates per test for three days. The Low, Mid, and High controls were also measured in two replicates per test, two tests per day for three days.

Sample	n	Mean (AU/mL)	Repeatability		Total Within-Laboratory Precision	
			SD	%CV	SD	%CV
Low Control	30	2.29	0.150	6.6	0.170	7.5
Mid Control	30	9.68	0.590	6.1	0.640	6.6
High Control	30	38.1	2.57	6.8	3.30	8.7
Sample 1	18	3.47	0.090	2.5	0.100	2.9
Sample 2	18	4.34	0.120	2.7	0.190	4.4
Sample 3	18	41.8	2.30	5.5	3.49	8.4
Sample 4	18	127	13.6	10.7	16.9	13.3

LOT-TO-LOT IMPRECISION

RBD ELISA - Lot-to-lot imprecision was determined by measuring four serum samples in two tests per day, three replicates per test for three days using two different lots of reagents. Positive and negative controls were also measured in two replicates per test, two tests per day for three days with two lots of reagents.

Sample	n	Mean (CI)	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	24	0.041	0	9.4	0	8.1	0	0	0	0	0.010	12.4
Positive Control	24	1.00	0.040	3.9	0	0	0	0	0	0	0.040	3.9
Sample 1	36	0.143	0.010	4.4	0.010	4.20	0.010	4.4	0.010	9.5	0.020	12.1
Sample 2	36	0.686	0.030	4.7	0.040	6.50	0.030	4.4	0.100	13.9	0.110	16.7
Sample 3	36	0.963	0.050	5.4	0.030	3.10	0.040	4.1	0.140	14.4	0.160	16.2
Sample 4	36	1.70	0.060	3.5	0.100	6.00	0.030	1.7	0.240	14.1	0.270	15.8

Spike ELISA - Lot-to-lot imprecision was determined by measuring three serum samples in two tests per day, three replicates per test for three days with two different lots of reagents. The Low, Mid, and High controls were also measured in two replicates per test, two tests per day for three days with two lots of reagents.

Sample	n	Mean (AU/mL)	Within-Run		Between-Run		Between- Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	66	2.23	0.140	6.2	0.080	3.4	0	0	0.060	2.8	0.170	7.6
Mid Control	66	9.80	0.510	5.2	0.300	3.0	0	0	0.100	1.0	0.600	6.1
High Control	66	38.3	3.13	8.2	0.790	2.1	1.50	3.9	0	0	3.56	9.3
Sample 1	36	3.46	0.130	3.6	0.120	3.5	0	0	0	0	0.170	5.0
Sample 2	36	4.29	0.130	3.0	0.130	3.0	0.020	0.5	0.050	1.1	0.190	4.4
Sample 3	36	41.8	3.07	7.3	1.09	2.6	3.41	8.1	0	0	4.71	11.3
Sample 4	36	122	12.6	10.4	8.64	7.1	7.58	6.2	2.77	2.3	17.3	14.2

SITE-TO-SITE REPRODUCIBILITY

RBD ELISA - Site-to-site reproducibility was determined by measuring four serum samples in two tests per day, three replicates per test for three days using at two different sites. Positive and negative controls were also measured in two replicates per test, two tests per day for three days at the two sites.

Sample	n	Mean (CI)	Within-Run		Between-Run		Between- Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	24	0.092	0.010	5.7	0.010	7.2	0	0	0.070	79.0	0.070	79.5
Positive Control	24	1.00	0.030	3.3	0	0	0	0	0	0	0.030	3.3
Sample 1	36	0.228	0.010	5.2	0.020	10.9	0	0	0.110	46.2	0.110	47.8
Sample 2	36	0.718	0.030	4.2	0.070	10.2	0	0	0.050	6.4	0.090	12.8
Sample 3	36	1.04	0.060	6.2	0.050	5.0	0.080	7.6	0	0	0.110	11.0
Sample 4	36	1.81	0.100	5.6	0.060	3.2	0.060	3.5	0.080	4.4	0.160	8.6

Spike ELISA - Site-to-site reproducibility was determined by measuring three serum samples in two tests per day, three replicates per test for three days at two different sites. The Low, Mid, and High controls were also measured in two replicates per test, two tests per day for three days at the two sites.

Sample	n	Mean (AU/mL)	Within-Run		Between-Run		Between- Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	42	2.23	0.150	6.9	0.080	3.8	0	0	0.15	6.7	0.230	10.4
Mid Control	42	9.54	0.550	5.7	0.350	3.7	0	0	0.310	3.2	0.720	7.5
High Control	42	38.8	3.86	10.0	0.720	1.8	1.23	3.2	1.36	3.5	4.33	11.2
Sample 1	36	3.54	0.160	4.6	0.270	7.6	0	0	0	0.0	0.310	8.9
Sample 2	36	4.52	0.150	3.4	0.450	9.9	0	0	0.190	4.1	0.510	11.3
Sample 3	33	42.5	2.54	6.0	1.28	3.0	2.04	4.8	0	0	3.50	8.2
Sample 4	35	138	13.58	9.9	15.1	11.0	0	0	15.12	11.0	25.31	18.4

ANALYTICAL SENSITIVITY

The analytical sensitivity - Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were established according to the recommendations in CLSI guideline EP17-A2. RBD ELISA and Spike ELISA summary data is presented below.

Sensitivity	RBD ELISA (CI)	Spike ELISA (AU/mL)
LoB	0.70	1.98
LoD	0.82	2.61
LoQ	——	3.20

LINEARITY

Linearity was demonstrated according to recommendations in CLSI guideline EP06-A. Three individual samples were proportionally diluted with negative serum samples. The negative serum samples used to make the dilutions were preCOVID-19 samples collected prior to September 2019.

The linear range is 3.1–160 AU/mL and the Analytical Measuring Range (AMR) is 3.2-160 AU/mL.

Sample	# Dilution Levels in the Linear Range	Linear Range (AU/mL)
1	11	8.2 - 145
2	11	4.2 - 161
3	10	3.1 - 72.1

CLINICAL SIGNIFICANCE

To evaluate the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of the COVID-SeroIndex, Kantaro Quantitative SARS-CoV-2 IgG Antibody IVD Kit, 92 positive samples and 284 negative samples were tested. These samples were all tested according to the IFU of the device. If the samples were negative on the RBD ELISA, they were not tested on the Spike ELISA. If they tested positive on the RBD ELISA, they were subsequently tested on the Spike ELISA.

Positive Percent Agreement:

For the positive samples confirmed with a known EUA-authorized molecular test, PPA was 97.8%. We note that two samples that tested negative with the COVID-SeroIndex Kantaro Quantitative SARS-CoV-2 IgG Antibody IVD Kit also tested negative on an existing EUA approved serology test, suggesting that these are true negative samples.

Days between Positive PCR and Sample Collection	Total Samples	Number Non-Reactive	Number Positive	PPA
≤ 7	0	0	0	N/A
8-14	1	0	1	100%
≥ 15	91	2	89	97.8%
Total	92	2	90	97.8%

Negative Percent Agreement:

For the negative samples, the NPA was 99.6%. There were 14 samples that tested positive on the RBD ELISA, below. Of these samples, 13 subsequently tested negative on the Spike ELISA, therefore the number of negative samples is 281 out of 282.

	Total Samples	Number Negative	Number Positive	NPA
PreCovid-19	272	271	1	99.6%
HIV Positive	10	10	0	100.0%
Total	282	281	1	99.6%

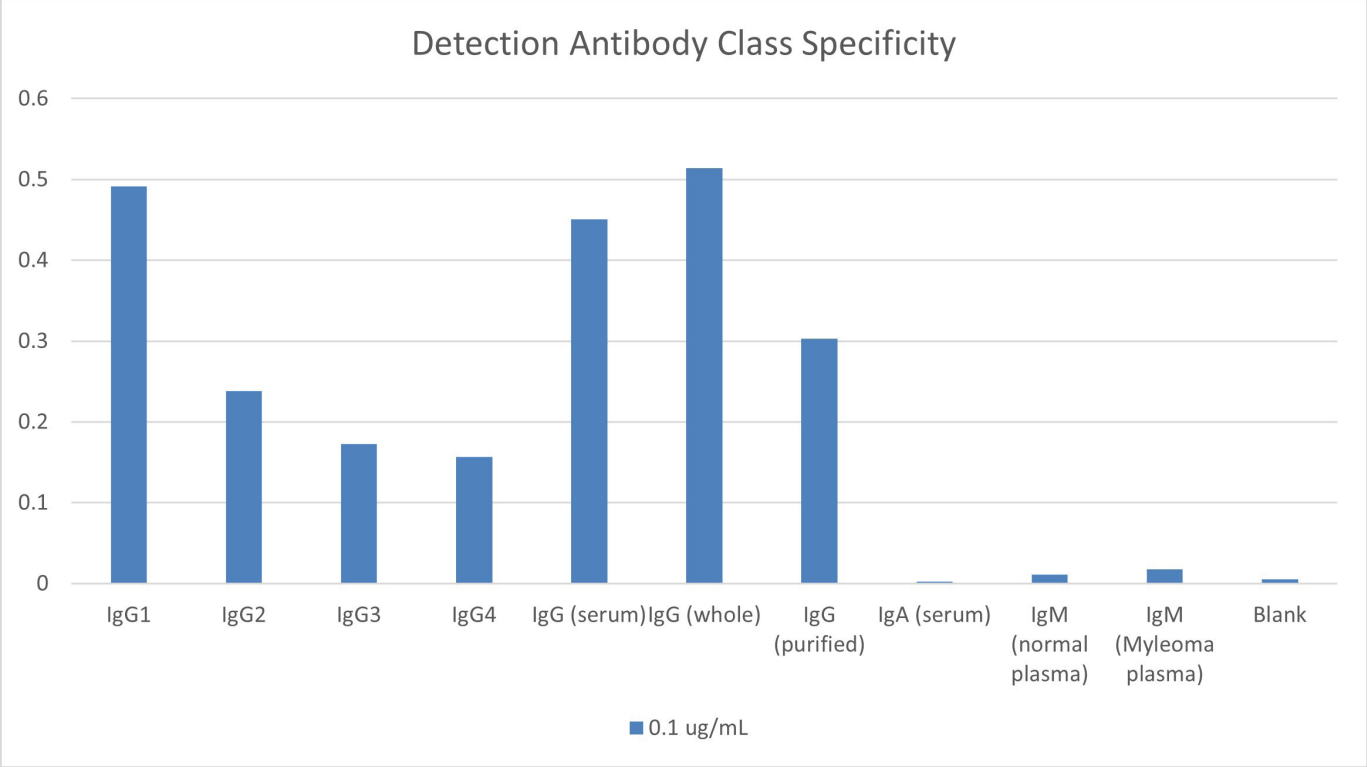
CALIBRATION

A monoclonal antibody with apparent viral neutralizing properties specific to the SARS-CoV-2 RBD of the Spike protein is used as a calibrator. This is used to generate a standard curve to convert OD units into arbitrary units per milliliter (AU/mL) in the Spike ELISA.

NIBSC Anti-SARS-CoV-2 Diagnostic Calibrant (20/162) approximate value (U) = 0.007 x Kantaro ELISA value (AU/mL).

CLASS SPECIFICITY

Class specificity of the monoclonal detection antibody was evaluated in an antigen-down ELISA study. Ten antigens, including seven different human IgG samples, were diluted to 25 ng/mL or 100 ng/mL (not shown) and coated on a plate. A dilution series of the monoclonal detection antibody was incubated on the plate prior to detection. Summary data indicates that the monoclonal detection antibody detects human IgG isotypes and has minimal detection of human IgA or IgM that approaches level of the blank with titration.



SPECIFICITY

Disease state samples collected prior to August 2019 were tested in this assay for cross-reactivity. No cross-reactivity was observed.

Disease State:

Antinuclear Antibody
Coronavirus HKU1
Coronavirus NL63
Coronavirus OC43
Coronavirus 229E
Cytomegalovirus
Epstein-Barr Virus
Hepatitis B Virus
Hepatitis C Virus
Herpes Simplex Virus
HIV
Human anti-Mouse Antibody
Influenza virus
Lupus
Rhuematoid Arthritis
Rheumatoid Factor
Rubella
Varicella Zoster Virus

INTERFERENCE

RBD ELISA:

Interference testing was performed following recommendations in CLSI guideline EP07-A3. Four serum samples were used to evaluate potential endogenous interferents. Data was evaluated quantitatively by comparing the percent difference between the mean CI value of the unspiked sample and the mean CI value of the spiked samples. All samples demonstrated a difference for the quantitative analysis of ≤ 15% at the specified concentration.

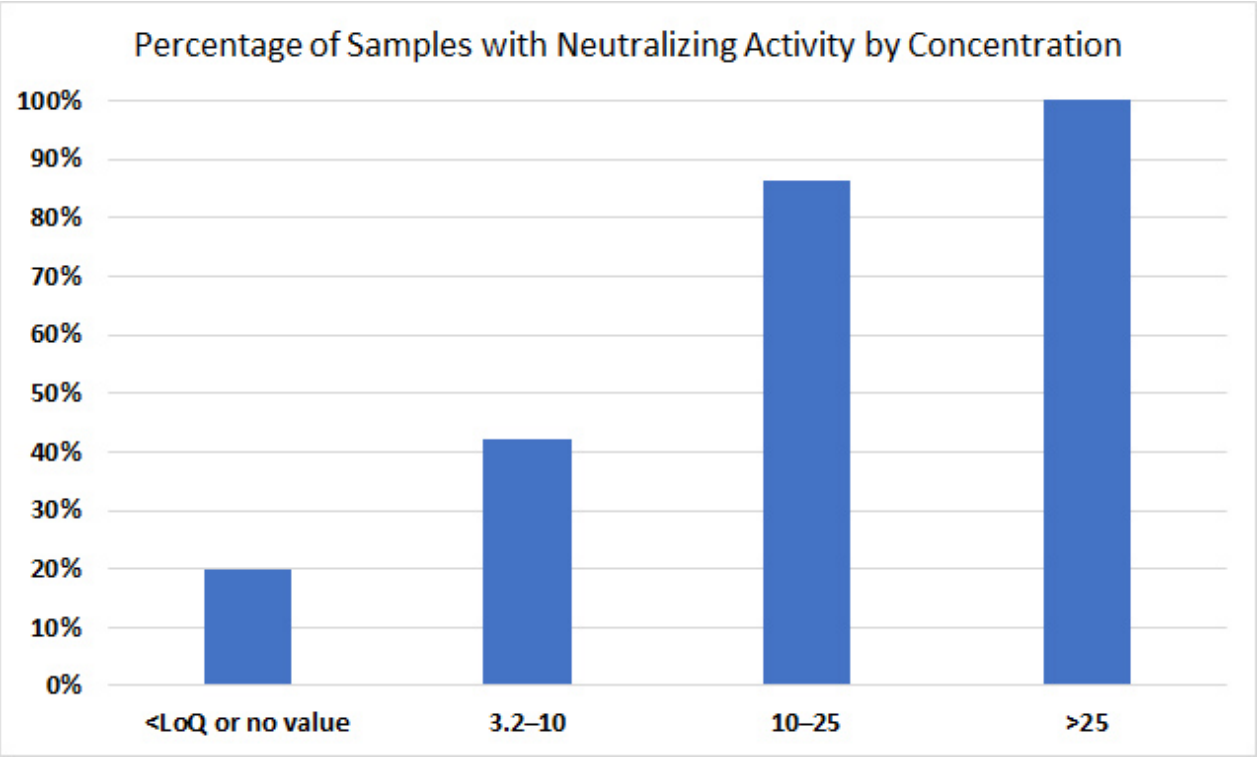
Spike ELISA:

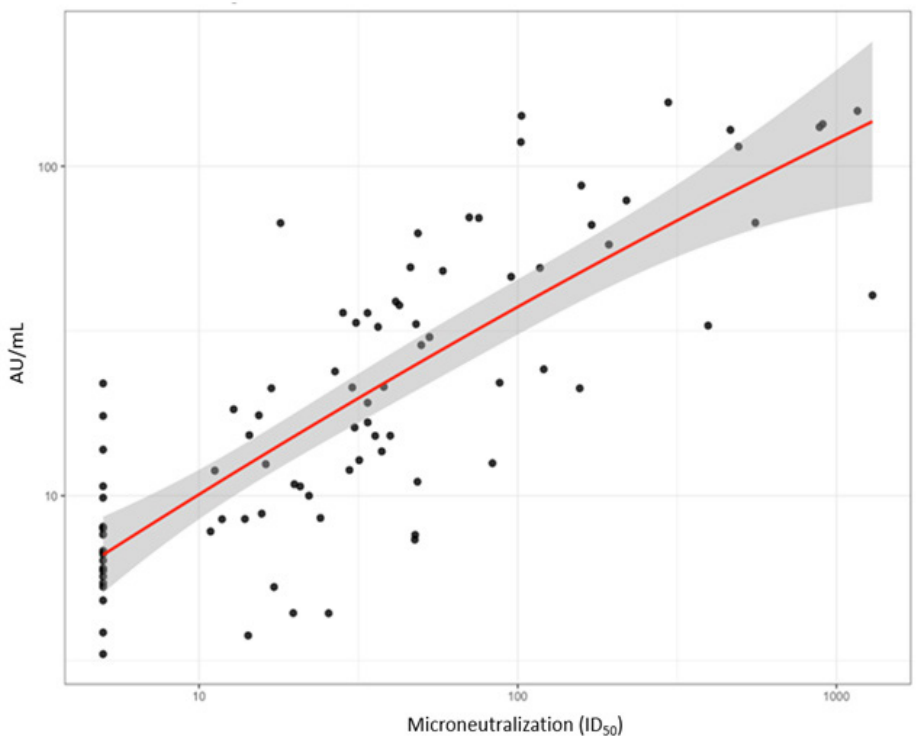
Interference testing was performed following recommendations in CLSI guideline EP07-A3. Two serum samples were used to evaluate potential endogenous interferents for the Spike ELISA, one at approximately 5.0 AU/mL, and one at approximately 50 AU/mL. Data was evaluated quantitatively by comparing the percent difference between the mean AU/mL value of the unspiked sample and the mean AU/mL value of the spiked samples. All samples demonstrated a difference for the quantitative analysis of ≤ 15% at the specified concentration.

Interferent	Highest Concentration
Conjugated Bilirubin	104 mg/dL
Unconjugated Bilirubin	96.6 mg/dL
Hemoglobin	10.6 g/dL
Total Protein	8.6 g/dL
Cholesterol	315 mg/dL
Triglycerides	6710 mg/dL

MICRONEUTRALIZATION

A study was conducted to correlate the quantitative levels of anti-Spike protein IgG antibodies to viral neutralization in a microneutralization (MN) assay. 120 patient samples with levels of antibodies across the AMR of the assay were evaluated in a MN assay. Values shown below are not multiplied by the dilution factor. Information on the format and interpretation of the MN assay can be found in the following reference: Amanat, F., *et. al*., "A Serological Assay to Detect SARS-CoV-2 Seroconversion in Humans"; Nature Medicine. 2020 May 12. PMID: 32398876.





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Powered by R&D Systems®

Catalog Number DSR200-CE

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