

COVID-SeroIndex

Kantaro Quantitative SARS-CoV-2 IgG Antibody RUO Kit

Powered by R&D Systems®

Catalog Number DSR200

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

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

**This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.**

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INTRODUCTION

COVID-SeroIndex, Kantaro Quantitative SARS-CoV-2 IgG Antibody RUO 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 (Heparin and EDTA) samples.

An initial ELISA is performed to test for antibodies reactive to the recombinant Receptor Binding Domain (RBD) of the SARS-CoV-2 Spike protein. A quantitative ELISA against the full length SARS-CoV-2 Spike protein is performed for positive specimens.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Samples must be pipetted within 15 minutes.
- 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.

PRINCIPLE OF THE 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 HINTS

- **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 # 896963) may contain a precipitate. Mix well prior to use.
- When mixing or reconstituting 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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	899269	4 plates	96 well polystyrene microplate coated with recombinant SARS-CoV-2 Spike protein RBD antigen.	Use a new Plate for each assay. Discard after use.
Spike Protein Microplate	899270	5 plates	96 well polystyrene microplate coated with full length recombinant SARS-CoV-2 Spike protein.	
RBD Conjugate Concentrate (1000X)	899056	1 vial	125 µL of 1000X concentrated monoclonal antibody specific to human IgG conjugated to horseradish peroxidase.	May be stored for up to 1 month at 2-8 °C.*
Spike Conjugate Concentrate (1000X)	899058	1 vial	125 µL of 1000X concentrated monoclonal antibody specific to human IgG conjugated to horseradish peroxidase.	
Conjugate Buffer - IgG ELISA	896962	1 bottle	120 mL of a protein based solution with preservatives.	
Sample Buffer - IgG ELISA	896963	3 bottles	91 mL of a buffered protein base with preservatives. <i>May contain a precipitate. Mix well prior to use.</i>	
TMB Substrate - IgG ELISA	895262	1 bottle	116 mL of stabilized hydrogen peroxide and chromogen (tetramethylbenzidine).	
Stop Solution - IgG ELISA	895263	1 bottle	116 mL of acidic solution.	
Wash Buffer - IgG ELISA	895264	2 bottles	101 mL of a 25-fold concentrated solution of buffered surfactant with preservative.	

* 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	83688	1 vial	1.0 mL of a monoclonal antibody in a protein buffered base with preservatives.	Store at 2-8 °C. Refer to vial label for expiration date.
RBD Negative Control	83689	1 vial	1.0 mL of a buffered protein base with preservatives.	
Spike Low Control	83690	1 vial	1.0 mL of a monoclonal antibody in a protein buffered base with preservatives.	
Spike Mid Control	83691	1 vial	1.0 mL of a monoclonal antibody in a protein buffered base with preservatives.	
Spike High Control	83692	1 vial	1.0 mL of a monoclonal antibody in a protein buffered base with preservatives.	
Spike Calibrator 1 (0 AU/mL)	83693	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives.	
Spike Calibrator 2 (0.82 AU/mL)	83694	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives.	
Spike Calibrator 3 (2.47 AU/mL)	83695	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives.	
Spike Calibrator 4 (7.41 AU/mL)	83696	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives.	
Spike Calibrator 5 (22.2 AU/mL)	83697	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives.	
Spike Calibrator 6 (66.7 AU/mL)	83698	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives.	
Spike Calibrator 7 (200 AU/mL)	83699	1 vial	1.25 mL of a monoclonal antibody in a buffered base with preservatives.	

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

OTHER SUPPLIES REQUIRED

- Plate sealers (R&D Systems®, Catalog # DY992)
- 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** microcentrifuge tubes for dilution of samples

WARNING & 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.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or 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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

Grossly hemolyzed or lipemic samples are not acceptable in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1X RBD Conjugate - For each plate, add 11 μ L of RBD Conjugate Concentrate (1000X) (part # 899056) to 11 mL of Conjugate Buffer (part # 896962). Mix well.

1X Spike Conjugate - For each plate, add 11 μ L of Spike Conjugate Concentrate (1000X) (part # 899058) to 11 mL of Conjugate Buffer (part # 896962). 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 # 895264) 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 # 896963) 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 ≤ -20 °C until use.

RBD Assay:

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

Spike Assay:

1. Dilute heat inactivated samples 5-fold in microcentrifuge tubes by adding 10 μ L of sample to 40 μ L of Sample Buffer.
2. Further dilute samples 40-fold (final 200-fold dilution) by adding 10 μ L of diluted sample from step 1 (diluted 5-fold) to 390 μ L 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 μ L of control (diluted 5-fold), heat inactivated sample (diluted 100-fold, run 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 μ L) using a squirt bottle, manifold dispenser, or autowasher. 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 μ L 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 μ L of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Protect from light.
6. Add 100 μ L 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, 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 # 83688, 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.

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

If the calculated CI value is ≥ 0.7 , 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-CoV2 Spike protein.

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	Medium	Medium
H	S1	S2	S10	S18	S26	S34	S42	S50	S58	S66	High	High

1. Add 100 μ L of control (diluted 5-fold), calibrator (undiluted), or RBD positive heat inactivated sample (diluted 200-fold, run 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 μ L) using a squirt bottle, manifold dispenser, or autowasher. 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 μ L 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 μ L of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Protect from light.
6. Add 100 μ L 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, 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*

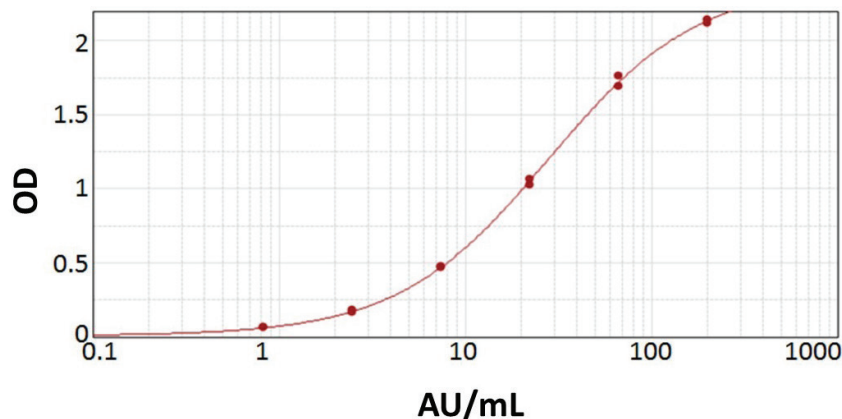
Spike ELISA Calculation of Results:

Create a standard curve by reducing the calibrator values using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the concentration versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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.

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

PRECISION

Intra-Assay Precision (Precision within an assay)

Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)

Three samples of known concentration were tested in separate assays to assess inter-assay precision.

RBD ELISA

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	3	3	3	18	18	18
Mean (CI)	0.565	0.914	1.58	0.616	0.862	1.53
Standard deviation	0.029	0.011	0.020	0.047	0.039	0.123
CV (%)	5.1	1.2	1.3	7.6	4.5	8.0

Spike ELISA

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	3	3	3	18	18	18
Mean (AU/mL)*	3.44	57.2	109	3.47	58.5	109
Standard deviation	0.080	3.03	7.49	0.093	5.09	12.7
CV (%)	2.3	5.3	6.9	2.7	8.7	11.7

*Spike values were not multiplied by the dilution factor.

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 and Spike ELISAs 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 blank serum samples. The blank 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 3.2-161 AU/mL.

Sample	# Dilution Levels in the Linear Range	Linear Range (AU/mL)
1	10	4.16-161
2	10	8.19-145
3	9	3.08-75.1

SAMPLE RESULTS

Samples known to be PCR positive for COVID-19 and samples obtained prior to September 2019 (PreCOVID-19) were tested according to the protocol on one lot of materials.

Sample Type	Total Samples	Number Negative	Number Positive
PreCOVID-19	470	469	1
PCR Positive	359	8	351

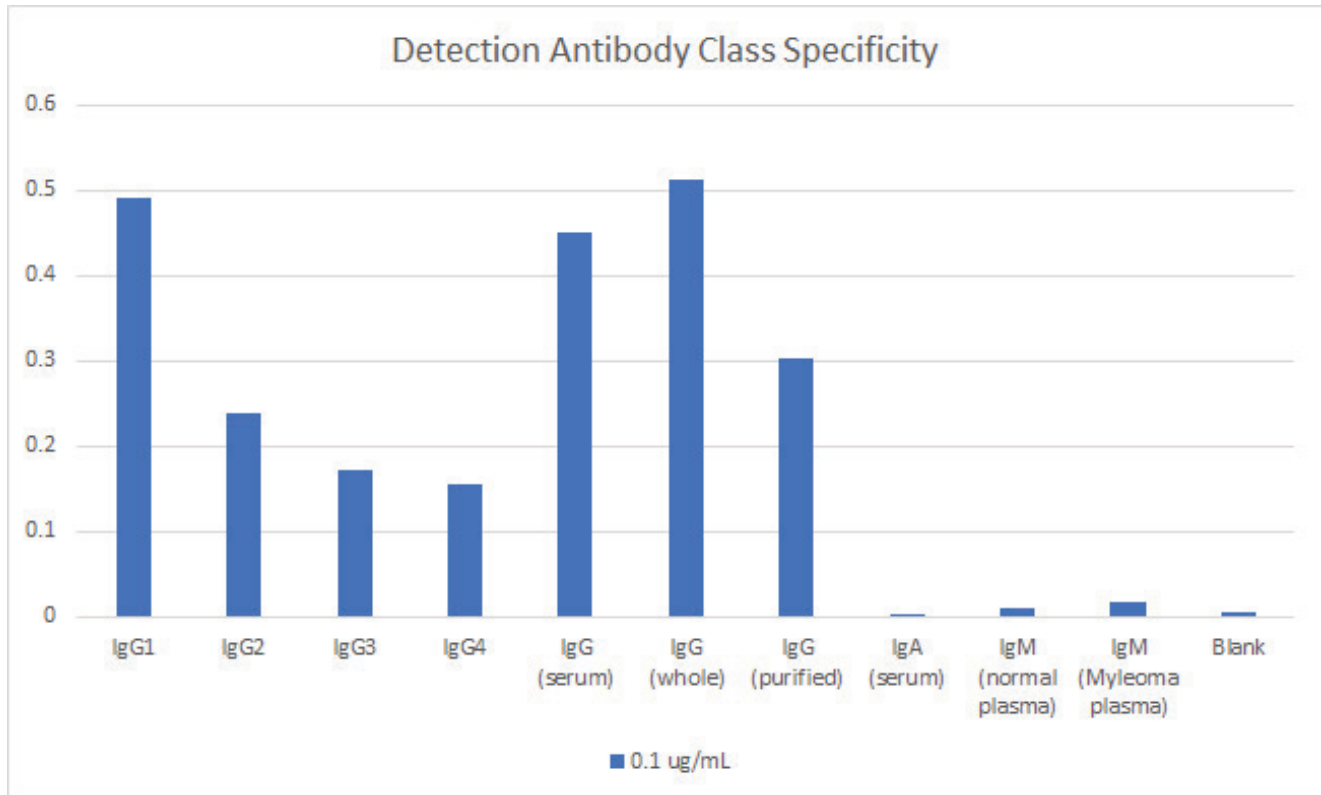
CALIBRATION

A monoclonal antibody with apparent viral neutralizing properties specific to the SARS-CoV-2 receptor binding domain 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.

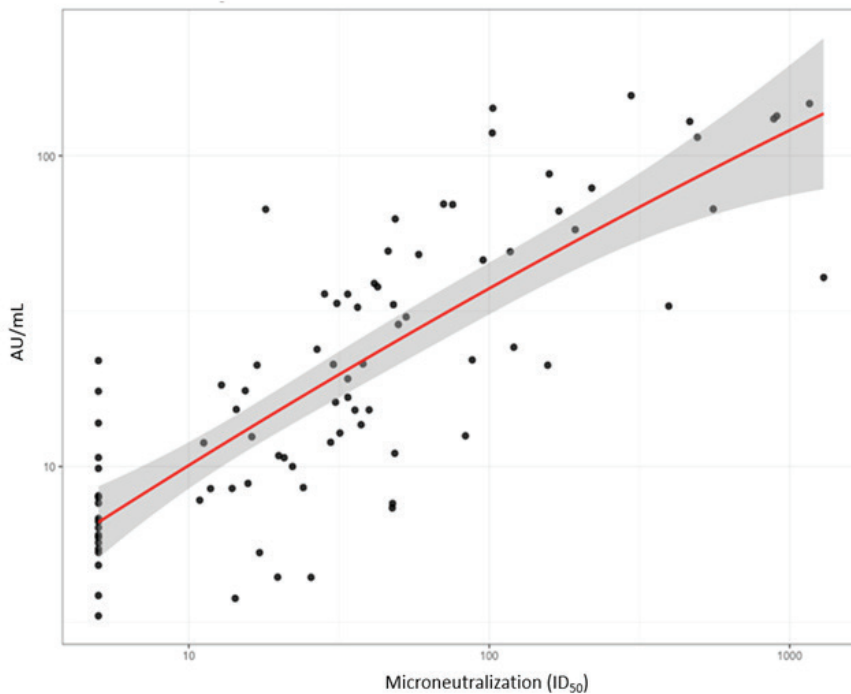
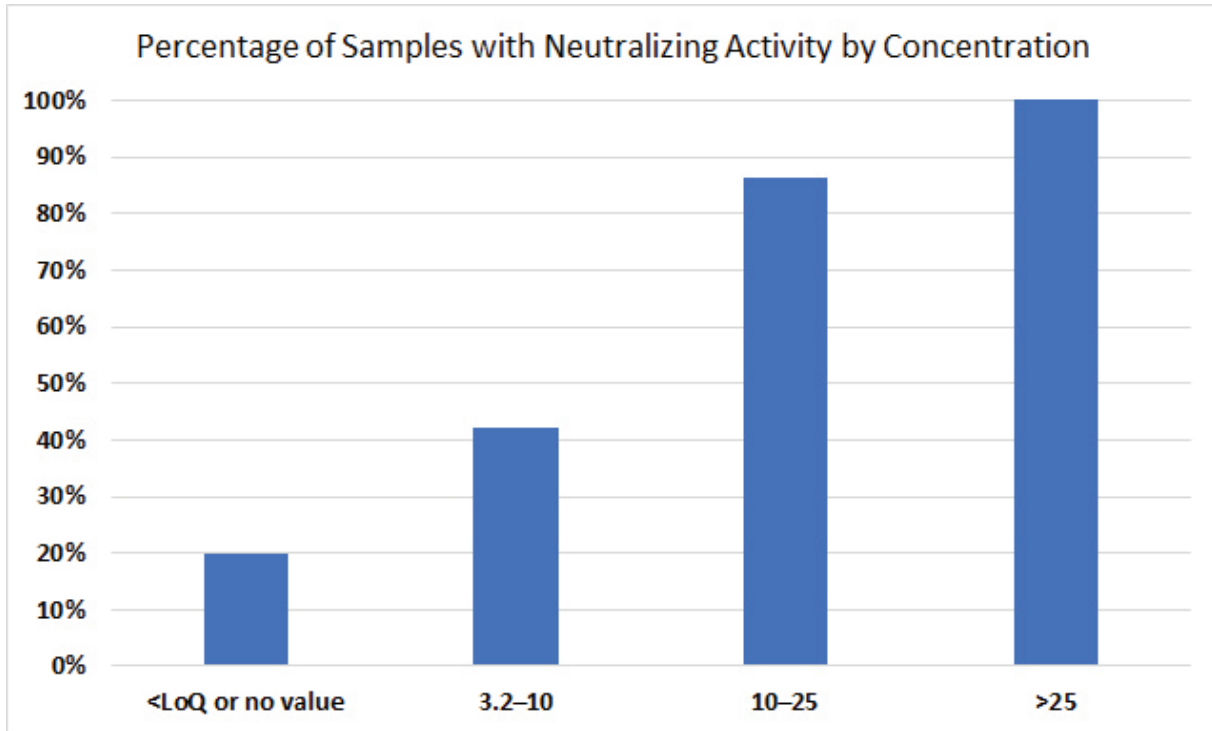
Disease State:

Antinuclear Antibody
Cold coronaviruses
Cytomegalovirus
Epstein-Barr Virus
Hepatitis B Virus
Hepatitis C Virus
Herpes Simplex Virus
HIV

Human anti-Mouse Antibody
Influenza virus
Lupus
Rheumatoid Arthritis
Rheumatoid Factor
Rubella
Varicella Zoster Virus

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.



ASSISTANCE AND CUSTOMER SUPPORT

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For e-mail support, contact info@bio-techne.com or techsupport@bio-techne.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Kantaro Bioscience LLC, our products, and our distributors can be found on our website kantarobio.com.

COVID-SeroIndex
Kantaro Quantitative SARS-CoV-2 IgG Antibody RUO Kit
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Catalog Number DSR200

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Patent Pending

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