COVID-SeroKlir

Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit

Powered by R&D Systems®

Catalog Number COV219

For qualitative and semi-quantitative detection of human IgG antibodies to the SARS-CoV-2 virus in serum and Li-Heparin plasma samples.

This kit contains sufficient materials to test 630 samples provided the assay is performed as described in this document.

- For Use Under Emergency Use Authorization Only
- For in vitro diagnostic use.
- For prescription use only.
- The results of this semi-quantitative test should not be interpreted as an indication or degree of immunity or protection from reinfection.

This package insert must be read in its entirety before using this product.

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INTENDED USE

The COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit is a two-step Enzyme-Linked Immunosorbent Assay (ELISA) intended for qualitative and semi-quantitative detection of human IgG antibodies to the SARS-CoV-2 virus in serum and Li-Heparin plasma. An initial qualitative ELISA step is performed against recombinant Receptor Binding Domain of SARS-CoV-2, followed, for positive specimens, by a confirmatory semi-quantitative ELISA step against full-length SARS-CoV-2 Spike protein. The COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit should not be used to diagnose acute SARS-CoV-2 infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform high complexity tests.

Results are for the qualitative and semi-quantitative detection of SARS-CoV-2 IgG antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

The sensitivity of the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

Samples should only be tested from individuals that are 15 days or more post symptom onset.

The COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

LIMITATIONS OF THE PROCEDURE

- For use under Emergency Use Authorization Only.
- For prescription use only.
- For *in vitro* diagnostic use only.
- The test should not be used to diagnose or exclude acute SARS-CoV-2 infection.
- Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.
- This test should only be used for testing samples collected 15 days after symptom onset. The performance of this test in samples collected less than 15 days after symptom onset has not been established.
- The clinical applicability of a semi-quantitative result is currently unknown and cannot be interpreted as an indication or degree of immunity nor protection from reinfection, nor compared to other SARS-CoV-2 antibody assays.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities as required.
- 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.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
- Positive results may be due to current or past infection with non-SARS-COV-2 corona virus strains, such as HKU1, NL63, OC43, or 229E.
- A negative result for an individual subject indicates the absence of detectable anti-SARS-CoV-2 antibodies. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies that are detected and are not present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
- Performance has only been established with specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
- Results obtained with this test may not be used interchangeably with values obtained by other manufacturers' tests.
- Not to be used to determine SARS-CoV-2 infection in donated blood units. This test should not be used for blood donor screening.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Recipients, and authorized labeling are available on the FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

Authorized laboratories using the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit ("your product" in the conditions below), must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

1. Authorized laboratories* using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media
2. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
3. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
4. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/ CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Kantaro Biosciences, Inc. (techsupport@bio-techne.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
6. All laboratory personnel using your product must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product.
7. Authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

*The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

PRINCIPLE OF ASSAY

The 2-step assay is an antigen-down enzyme immunoassay which utilizes a recombinant SARS-CoV-2 Receptor Binding Domain (RBD) antigen pre-coated onto a 96-well microplate in step 1. When the sample is added, antibodies found in the sample that recognize SARS-CoV-2 RBD antigen 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. 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 in the first step ELISA and tested in the 2nd step ELISA, the Spike ELISA.

Positive samples from step 1 are evaluated on a second orthogonal ELISA that provides semi-quantitative measurement of IgG antibodies to the full-length 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 RECOMMENDATIONS

- 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.
- 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.
- 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 after it is added to the plate.
- 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	7 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. Unused plates from open kits may be stored for up to 1 month at 2-8 °C
Spike Protein Microplate	899270	3 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.* Discard diluted solutions after use.
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 buffered protein base with preservatives.	
Sample Buffer - IgG ELISA	896963	3 bottles	91 mL of a buffered protein base with preservatives.	
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 monoclonal antibody in a protein buffered base with preservatives.	Store at 2-8 °C. Refer to vial label for expiration date.* Discard diluted solutions after use.
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 monoclonal antibody in a protein buffered base with preservatives.	
Spike Mid Control	83691	1 vial	1.0 mL of monoclonal antibody in a protein buffered base with preservatives.	
Spike High Control	83692	1 vial	1.0 mL of 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

- 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)

SAFETY & 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 acidic 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.
- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA that meet requirements to perform
- high-complexity tests.
- This test has been authorized only for the presence of IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

TABLE OF SYMBOLS

SYMBOL	MEANING
	Manufacturer's Catalog designation or number
	Use by, expiration date
	Lot Number
	*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 restricts this device to sale by or on the order of a licensed healthcare practitioner
	Intended Use

SAMPLE COLLECTION & STORAGE

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 2-8 °C or ≤ -20 °C.

Plasma - Collect plasma using 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 2-8 °C or ≤ -20 °C.

Serum and Li-Heparin plasma samples are stable for 7 days at 2-8 °C and 3 weeks at ≤ -20 °C. Do not freeze samples more than one time prior to use.

REAGENT PREPARATION

1X RBD Conjugate - For each plate, add 11 µL 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 µL 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 2-8 °C or ≤ -20 °C until use.

RBD ELISA Step:

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.

INTERPRETATION OF RESULTS

COVID-SeroKlir Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit Interpretation of Results

<table>
<tr><th colspan="6">COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit</th></tr>
<tr><th colspan="2">RBD ELISA
(Qualitative)</th><th colspan="2">Spike ELISA
(Semi-Quantitative)</th><th rowspan="2">Final Result</th><th rowspan="2">Description</th></tr>
<tr><th>CI Value</th><th>Result</th><th>AU/mL Value</th><th>Result</th></tr>
<tr><td>< 0.7</td><td>Negative</td><td>N/A</td><td>N/A</td><td>Negative</td><td>IgG antibodies to SARS-CoV-2 are NOT detected.</td></tr>
<tr><td rowspan="3">≥ 0.7</td><td rowspan="3">Positive.
Additional Test is Required with Spike ELISA.</td><td>< 3.2</td><td>Negative</td><td rowspan="3">Positive</td><td>IgG antibodies to SARS-CoV-2 ARE detected.
The measured value in AU/mL is reported.</td></tr>
<tr><td>Between 3.2 and 125</td><td>Positive</td><td>IgG antibodies to SARS-CoV-2 ARE detected.
The measured value is reported as “>125 AU/mL”.</td></tr>
<tr><td>> 125</td><td>Positive</td></tr>
</table>

In the RBD ELISA step, 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 < 0.70, the sample is determined to be negative. See Interpretation of Results table above.

The Spike ELISA step reports results in arbitrary units per milliliter (AU/mL). A value less than the Limit of Quantitation (LoQ) is considered to be negative. The LoQ is 3.2 AU/mL. A value greater than 3.2 AU/mL is considered positive. In addition to reporting a value greater than 3.2 AU/mL as positive, the measured AU/mL can be reported. Values greater than the analytical measuring range should be reported as >125 AU/mL. See Interpretation of Results table above.

COVID-SEROKLIR, KANTARO SEMI-QUANTITATIVE SARS-COV-2 IGG ANTIBODY KIT ASSAY PROCEDURE

STEP 1: RBD ELISA 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, tested in singlets), or sample buffer (blank) per well. Incubate for 2 hours at room temperature on benchtop. Cover with an adhesive strip if needed.
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 automated plate washer. Complete removal of liquid at each step is essential for 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 is required to correct for optical imperfections in the plate.

STEP 1: RBD ELISA ASSAY *CONTINUED*

RBD 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 control.

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.

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

RBD Quality Control:

Controls with known anti-SARS-CoV-2 IgG concentrations (provided) should be tested in each plate. Satisfactory performance is obtained when controls fall within the established ranges provided in the Certificate of Analysis.

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

If the results obtained for the controls and blank do not fall within the acceptable limits, the assay results are invalid and samples should be run again.

STEP 2: SPIKE ELISA 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	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
B	Cal 2	Cal 2	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
C	Cal 3	Cal 3	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
D	Cal 4	Cal 4	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
E	Cal 5	Cal 5	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
F	Cal 6	Cal 6	S7	S7	S15	S15	S23	S23	S31	S31	Low	Low
G	Cal 7	Cal 7	S8	S8	S16	S16	S24	S24	S32	S32	Mid	Mid
H	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	High	High

1. Add 100 µL of control (diluted 5-fold), calibrator (undiluted), or RBD positive heat inactivated sample (diluted 200-fold, tested in duplicates) per well. Incubate for 2 hours at room temperature on benchtop. Cover with an adhesive strip if needed.
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 automated plate 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.

STEP 2: SPIKE ELISA 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, control, and sample.

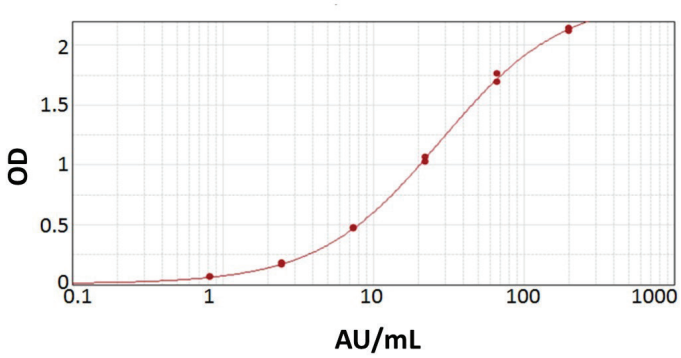
The calibrators are provided as individual vials and are ready to use. 100 µL of each calibrator is added to the calibrator wells and processed along with the unknown samples and controls.

A monoclonal antibody that recognizes the SARS-CoV-2 RBD of the Spike protein is used as a calibrator. This is used to generate a calibration curve to convert corrected OD units into arbitrary units per milliliter (AU/mL) in the Spike ELISA.

Seven discrete calibrators are provided with the concentrations listed in the following table. Calibration of the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit is traceable to an in-house value assigned reference calibrator.

Assigned Calibrator Values:
Each calibrator is measured in duplicate and the average of the two measurements is used to generate the calibration curve. A four-parameter logistic (4-PL) curve is fitted to the calibrator values to convert corrected OD units to AU/mL. The calibration curve is generated by the end-user for every plate using appropriate software (e.g., SoftMax Pro, Prism). There is no software provided with the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit.

The four-parameter curve must have a correlation coefficient of > 0.98 or the assay is rejected.



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

Example data only.

Typical calibration curve: A 7-point standard curve is generated by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve fit.

The mean value of the duplicate measured OD value of unknown specimens is used to calculate concentration by the following equation where X = AU/mL, Y = OD450, A =lower asymptote, B = hill slope, C = IC50, D = upper asymptote of 4PL curve. These values and the conversion of unknown samples values from the corrected OD to AU/mL are calculated with appropriate software (e.g., SoftMax Pro, Prism GraphPad).

$$X=C\left(\frac{A-Y}{Y-D}\right)^{\left(\frac{1}{B}\right)}$$

Spike Quality Control:
Satisfactory performance is obtained when controls fall within the established ranges provided in the Certificate of Analysis. If the results obtained do not fall within the acceptable limits, the assay results are invalid and samples should be retested.

PERFORMANCE CHARACTERISTICS

ANALYTICAL MEASURING RANGE

The RBD ELISA step is a qualitative ELISA and there is no defined analytical measuring range (AMR). The output of tthis step 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 positive control. Patient samples with a CI ≥ 0.70 for the RBD ELISA step are tested in the Spike ELISA step. Patient samples with a CI < 0.70 for the RBD ELISA. are considered negative and not tested in the Spike ELISA step.

The AMR for the Spike protein ELISA step is based on the Limit of Blank, Limit of Detection, Limit of Quantitation (LoQ), Linearity, and Precision studies conducted. 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 section, and the high calibrator, which is set at 200 AU/mL. Results of the semi-quantitative Spike ELISA step are reported in arbitrary units per milliliter (AU/mL).

The claimed AMR for the Spike ELISA is 3.2-125 AU/mL.

WITHIN-SITE IMPRECISION

RBD ELISA - Within Laboratory Precision for the RBD ELISA step 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.

Lot-to-lot imprecision for the RBD ELISA step was determined by measuring four serum samples in two runs per day, three replicates per run for three days using two different lots of reagents. Positive and negative controls were also measured in two replicates per run, two runs 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	9.40%	0	8.10%	0	0.00%	0	0.00%	0.01	12.40%
Sample 1 (Negative)	36	0.143	0.01	4.40%	0.01	4.20%	0.01	4.40%	0.01	9.50%	0.02	12.10%
Sample 5 (Negative)	36	0.173	0.01	5.50%	0.01	8.00%	0.01	7.30%	0.03	17.80%	0.04	21.50%
Sample 6 (Near Cutoff)	36	0.597	0.03	4.70%	0.06	10.90%	0.01	1.80%	0.08	12.90%	0.11	17.60%
Sample 2 (Near Cutoff)	36	0.686	0.03	4.70%	0.04	6.50%	0.03	4.40%	0.1	13.90%	0.11	16.70%
Sample 7 (Near LoD)	36	0.75	0.04	5.60%	0.04	4.70%	0.03	4.60%	0.13	16.90%	0.14	19.00%
Sample 3 (Near LoD)	36	0.963	0.05	5.40%	0.03	3.10%	0.04	4.10%	0.14	14.40%	0.16	16.20%
Positive Control	24	1	0.04	3.90%	0	0.00%	0	0.00%	0	0.00%	0.04	3.90%
Sample 4 (Positive)	36	1.704	0.06	3.50%	0.1	6.00%	0.03	1.70%	0.24	14.10%	0.27	15.80%
Sample 8 (Positive)	36	1.864	0.07	3.80%	0.04	2.20%	0.1	5.20%	0.27	14.30%	0.29	15.80%

WITHIN-SITE IMPRECISION *CONTINUED*

Spike ELISA - Within Laboratory Precision for the Spike ELISA step 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.

Lot-to-lot imprecision for the Spike ELISA step was determined by measuring three serum samples in two runs per day, three replicates per run for three days with two different lots of reagents. The Low, Mid, and High controls were also measured in two replicates per run, two runs 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
High Ctrl	66	38.28	3.13	8.2%	0.79	2.1%	1.50	3.9%	0.00	0.0%	3.56	9.3%
Low Ctrl	66	2.23	0.14	6.2%	0.08	3.4%	0.00	0.0%	0.06	2.8%	0.17	7.6%
Mid Ctrl	66	9.80	0.51	5.2%	0.30	3.0%	0.00	0.0%	0.10	1.0%	0.60	6.1%
Sample 353	36	3.46	0.13	3.6%	0.12	3.5%	0.00	0.0%	0.00	0.0%	0.17	5.0%
Sample 534	36	4.29	0.13	3.0%	0.13	3.0%	0.02	0.5%	0.05	1.1%	0.19	4.4%
Sample 735	36	41.80	3.07	7.3%	1.09	2.6%	3.41	8.1%	0.00	0.0%	4.71	11.3%
Sample 744	36	58.47	4.55	7.8%	1.03	1.8%	3.68	6.3%	0.00	0.0%	5.95	10.2%
Sample 820	36	121.83	12.64	10.4%	8.64	7.1%	7.58	6.2%	2.77	2.3%	17.31	14.2%
Sample 850	36	104.19	10.32	9.9%	5.13	4.9%	9.65	9.3%	1.25	1.2%	15.08	14.5%

SITE-TO-SITE REPRODUCIBILITY

RBD ELISA - Site-to-site reproducibility for RBD ELISA step 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	0.01	5.7%	0.01	7.2%	0.00	0.0%	0.07	79.0%	0.07	-
Positive Control	24	1.000	0.03	3.3%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.03	3.3%
Sample 1 (Negative)	36	0.228	0.01	5.2%	0.02	10.9%	0.00	0.0%	0.11	46.2%	0.11	47.8%
Sample 2 (Near Cutoff)	36	0.718	0.03	4.2%	0.07	10.2%	0.00	0.0%	0.05	6.4%	0.09	12.8%
Sample 3 (Near LoD)	36	1.044	0.06	6.2%	0.05	5.0%	0.08	7.6%	0.00	0.0%	0.11	11.0%
Sample 4 (Positive)	36	1.811	0.10	5.6%	0.06	3.2%	0.06	3.5%	0.08	4.4%	0.16	8.6%
Sample 5 (Negative)	36	0.299	0.02	7.9%	0.02	8.2%	0.01	2.9%	0.15	48.6%	0.15	50.0%
Sample 7 (Near LoD)	36	0.832	0.05	6.0%	0.02	2.7%	0.06	7.2%	0.00	0.0%	0.08	9.8%
Sample 8 (Positive)	36	2.032	0.08	3.8%	0.07	3.5%	0.09	4.5%	0.00	0.0%	0.14	6.9%
Sample 8 (Positive)	36	1.864	0.07	3.8%	0.04	2.2%	0.10	5.2%	0.27	14.3%	0.29	15.8%

Spike ELISA - Site-to-site reproducibility for the Spike ELISA step 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
High Ctrl	42	38.79	3.86	10.0%	0.72	1.8%	1.23	3.2%	1.36	3.5%	4.33	11.2%
Low Ctrl	42	2.23	0.15	6.9%	0.08	3.8%	0.00	0.0%	0.15	6.7%	0.23	10.4%
Mid Ctrl	42	9.54	0.55	5.7%	0.35	3.7%	0.00	0.0%	0.31	3.2%	0.72	7.5%
Sample 353	36	3.54	0.16	4.6%	0.27	7.6%	0.00	0.0%	0.00	0.0%	0.31	8.9%
Sample 534	36	4.52	0.15	3.4%	0.45	9.9%	0.00	0.0%	0.19	4.1%	0.51	11.3%
Sample 735	33	42.45	2.54	6.0%	1.28	3.0%	2.04	4.8%	0.00	0.0%	3.50	8.2%
Sample 744	36	63.67	7.37	11.6%	0.00	0.0%	2.35	3.7%	7.04	11.1%	10.46	16.4%
Sample 820	35	137.71	13.58	9.9%	15.09	11.0%	0.00	0.0%	15.12	11.0%	25.31	18.4%
Sample 850	36	99.59	12.96	13.0%	0.00	0.0%	2.37	2.4%	12.65	12.7%	18.26	18.3%

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. The summary data for the RBD and Spike ELISA steps 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 serum samples were proportionally diluted with negative serum samples. The negative serum samples used to make the dilutions were pre-COVID samples collected prior to September 2019. Each dilution was measured with four replicates.

The linear range was demonstrated to be 1.89-125.7 AU/mL with a deviation from linearity ≤ 15%. The Analytical Measuring Range (AMR) is 3.2-125 AU/mL.

CLINICAL AGREEMENT STUDY

To evaluate clinical performance of the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit a clinical study using retrospective samples was conducted. Positive Percent Agreement (PPA) was determined by evaluating 251 unique serum samples collected from SARS-CoV-2 positive subjects confirmed by RT-PCR. Negative Percent Agreement (NPA) was determined by evaluating 284 negative serum samples. The 284 negative serum samples were collected prior to December 2019 (Pre-COVID) and 11 of the samples were collected from HIV-positive subjects.

Positive Percent Agreement:

Out of the 251 PCR positive subjects, 235 subjects had the days from symptoms onset information available. The PPA was calculated considering days from PCR result and also from symptoms onset, as indicated in the following two tables.

Positive Percent Agreement in PCR-Confirmed Positive Subjects from Days from Positive PCR Test				
Days between Positive PCR and Sample Collection	Total Samples	Number Positive	PPA	95% Confident Interval
≤ 7	3	3	100%	43.85% - 100%
8-14	3	3	100%	43.85% - 100%
≥ 15	245	242	98.78%	96.46% - 99.58%
Total	251			

Positive Percent Agreement in PCR-Confirmed Positive Subjects from Days from Symptom Onset				
Days between Symptom Onset and Sample Collection	Total Samples	Number Positive	PPA	95% Confident Interval
≤ 7	0	0	N/A	N/A
8-14	0	0	N/A	N/A
≥ 15	235	233	99.15%	96.95% - 99.77%
Total	235			

Negative Percent Agreement:

For the negative samples, the NPA was 99.6% (283/284, 95% CI: 98.0%-100%). All 11 of the HIV positive samples tested negative with the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit.

	Total Samples	Number Negative	NPA	95% Confidence Interval
PreCOVID-19	273	272	99.6% (272/273)	97.5% to 99.9%
HIV Positive	11	11	100.0% (11/11)	74.12% to 100%
Total	284	283	99.6% (283/284)	98.0% to 99.9%

ANALYTICAL SPECIFICITY

POTENTIAL CROSS-REACTIVITY

The COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit was evaluated for potential cross-reactivity. Eighty-six (86) serum samples with antibodies to the diseases included in the table below were tested with the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit. The samples evaluated were all collected prior to August 2019. No cross-reactivity was observed.

Potential Cross-Reactivity Results for the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit

Disease	n	% Negative
Epstein-Barr Virus	5	100%
Rheumatoid Arthritis	5	100%
Rheumatoid Factor	5	100%
Cytomegalovirus	5	100%
Human anti-Mouse Antibody	5	100%
Varicella Zoster Virus	5	100%
Rubella	5	100%
Antinuclear Antibody	5	100%
Influenza B	5	100%
Herpes Simplex Virus	5	100%
Hepatitis C Virus	5	100%
Hepatitis B Virus	5	100%
Lupus	5	100%
HIV	11	100%
Common Coronavirus	10	100%
Total	86	100%

POTENTIAL INTERFERING SUBSTANCES

Potential interference with the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit test was evaluated by assessing potential interference with the RBD ELISA step as well as the Spike ELISA step.

RBD ELISA Step:
Interference testing was conducted 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 sample without the potential interferent substance and the mean CI value of the samples containing the potential interferent substance. All samples demonstrated a difference of ≤ 15% at the specified concentrations in the table below.

Spike ELISA Step:
Interference testing was conducted 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 by comparing the percent difference between the mean AU/mL value of the sample without the potential interferent substance and the mean AU/mL value of the samples containing the potential interferent substance. All samples demonstrated a difference of ≤ 15% at the specified concentration.

The following potential interferent were evaluated at the indicated concentrations:

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

MATRIX EQUIVALENCY

Matrix equivalency between serum and Lithium Heparin plasma specimens was evaluated with the COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit by assessing equivalence between serum and lithium heparin plasma for the RBD ELISA step as well as the Spike ELISA step.

RBD ELISA Step:

Matrix equivalency for the RBD ELISA step was demonstrated by testing 36 matched serum and Li-Heparin plasma samples that spanned the range of expected CI values. Passing-Bablok regression analysis was used to compare the results from the serum sample measurement to the results from the Li-Heparin plasma sample measurements. The table below summarizes the results of this study.

Comparison	n	Range (CI)	Slope (95% CI)	Intercept (95% CI)	r
Serum vs Li-Heparin Plasma	36	0.13–5.1	1.02 (0.97–1.07)	-0.01 (-0.03–-0.05)	0.99

Spike ELISA Step:

Matrix equivalency for the Spike ELISA step was demonstrated by testing 18 matched serum and Li-Heparin plasma samples that spanned the analytical measuring range of the assay. Passing-Bablok regression analysis was used to compare the results from the serum sample measurement to the results from the Li-Heparin plasma sample measurements. The table below summarizes the results of this study.

Comparison	n	Range (CI)	Slope (95% CI)	Intercept (95% CI)	r
Serum vs Li-Heparin Plasma	18	6.1-96.4	0.99 (0.96–1.06)	-0.04 (-1.8–0.88)	0.99

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COVID-SeroKlir
Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit
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Catalog Number COV219

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