AKSTON BIOSCIENCES

Akston Biosciences AntiCoV-ID™ IgG ELISA

Instructions for Use

600016

Reagents for 80 Determinations

IVD Rx ONLY
for *in vitro* diagnostic and Laboratory Professional Use

Manufactured by:
Akston Biosciences Corporation
100 Cummings Center
Suite 454C
Beverly, MA 01915
United States

The AntiCoV-ID™ IgG ELISA test kits are provided for use only by laboratories certified to perform high complexity testing, and they are not to be used for at-home testing. The kits are distributed in the United States under Section IV.D of the Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency and while the AntiCoV-ID™ IgG ELISA EUA application is under review by FDA. In accordance with FDA guidance:

- This test has not been reviewed by the FDA.
- Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.
- Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- Not for screening of donated blood.

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Explanation of Symbols Used on Labels

	Reagents for 80 determinations
	Retest/Expiry/Use by date
	Storage temperature range
	Catalog number
	Lot number
	In vitro diagnostic device
	Read instructions before use
	Keep away from sunlight
	Date of manufacture
	Manufacturer
	Caution

Intended Use

The Akston Biosciences AntiCoV-ID™ IgG ELISA is a method for the quantitative and qualitative determination of anti-SARS-CoV-2 spike protein receptor binding domain (RBD) IgG antibodies in human serum, citrate plasma or EDTA plasma.

The AntiCoV-ID™ IgG ELISA is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior 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, to perform high complexity tests. Results are for the detection of SARS-CoV-2 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 positive results to the appropriate public health authorities. 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 may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

Summary and Explanation of the Assay

The SARS-CoV-2 virus is a highly contagious pathogen, and it has been implicated in a worldwide pandemic. The SARS-CoV-2 virus is responsible for the disease it causes, namely the Coronavirus Disease 2019 (COVID-19), and as such the virus is also known as the COVID-19 virus or the 2019 Novel Coronavirus. COVID-19 is associated with symptoms such as fever, tiredness, dry cough, aches and pains, nasal congestion, runny nose, and sore throat. In some cases, the host may exhibit mild symptoms or may be asymptomatic. However, more severe cases are associated with severe respiratory distress, pneumonia, and even death.

SARS-CoV-2 is of the genus Betacoronavirus. Coronavirus contains four protein structures, including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. Among them, S protein is principally involved in the attachment and entry of the virus into cells. Entry is thought to be accomplished by binding to human ACE2 receptor via the S protein receptor-binding domain (RBD). Therefore, due to its critical role on cell entry, the SARS-CoV-2 spike protein RBD has emerged as a strong target for the development of virus attachment inhibitors, neutralizing antibodies, and vaccines (Jun Lan, et al. *Nature* 2020).

There is an urgent need to understand which portion of the population may already have developed endogenous IgG-type antibodies against the virus, to support convalescent plasma clinical trials by identifying those with IgG-type antibodies, and to identify which therapies perform well in vaccination trials against SARS-CoV-2. Moreover, due to the vast scale of the pandemic, there is an urgent need to obtain this data in a quantitative and high-throughput fashion in order understand differences in the antibody titers between patients.

Underlying Principle of the Procedure

Akston Biosciences' AntiCoV-ID™ IgG ELISA is an indirect, enzyme linked immunosorbent assay (ELISA) designed to measure anti-spike protein receptor binding domain (RBD) IgG antibodies against the SARS-CoV-2 virus (COVID-19 virus, 2019 Novel Coronavirus) in human patient serum and plasma samples, including heat-inactivated serum or heat-inactivated plasma. The indirect immunoassay uses a recombinant SARS-CoV-2 spike protein RBD immobilized on ELISA plates as the capture antigen to bind the SARS-CoV-2 specific anti-spike RBD antibodies in the serum samples when incubated in the microplate wells. A simple wash step removes all unbound proteins, leaving the anti-SARS-CoV-2 spike protein RBD antibodies bound to the plate. A second incubation is performed where the anti-spike protein RBD antibodies are detected by an anti-human IgG antibody (not cross-reactive to IgM) conjugated to horseradish peroxidase (HRP). After a second simple wash step to remove the unbound enzyme-conjugate, the assay plate wells are incubated with 3,3',5,5'-trimethylbenzidine which causes a colorimetric change that is proportional to the amount of bound enzyme conjugate in each well. The color development is stopped by adding acid that halts development, and the color density of each well is measured using a spectrophotometric microplate reader.

Precautions and Warnings

- For *in vitro* diagnostic use. None of the AntiCoV-ID™ IgG ELISA test kit reagents are for internal or external use in animals or people.
- Follow all precautions for storing, using, and disposing of hazardous materials at your site. Assay Stop Solution contains 1% sulfuric acid. Some of the reagents contain preservatives in non-declarable concentrations. Avoid eye and skin contact with samples and reagents. In case of eye or skin contact, flush with copious amounts of water. Remove and wash contaminated clothing. In case of ingestion, obtain medical advice.
- Caution: Federal law restricts this device to sale by or on the order of a physician.
- Use universal precautions when handling patient blood, serum, plasma, and other biospecimens. All blood, serum, and plasma samples should be treated as potentially infectious. The product must only be used by trained laboratory personnel in a clinical or research laboratory.
- If the packed reagents are visibly damaged, do not use the test kit.
- Before using the product, read the Instructions for Use carefully. Use only the valid version provided with the product.
- The pipetting volumes, incubation times, temperatures, and preparation steps given in the Instruction for Use must be adhered to.
- Do not substitute or mix AntiCoV-ID™ IgG ELISA test kit reagents with reagents from other manufacturers.
- Observe Good Laboratory Practice (GLP) and safety guidelines.
- The kit is designed such that each well is only used one time.

Required and Optional Materials That Are Not Provided

- Calibrated single channel (e.g. 10 µL, 100 µL, 200 µL and 1000 µL), multichannel pipettes (e.g. 10 µL, 100 µL and 300 µL) and pipette tips of appropriate sizes. Use of multichannel pipettes is encouraged for decreasing the overall assay time
- Items for preparation of reagents (e.g. 1L and 2L graduated cylinders), mixing containers and tubes (e.g. 15 mL and 50 mL tubes, and 2 L bottles)
- Microplates or tubes (e.g. 0.5 mL or 1.5 mL tubes) for performing dilutions of serum samples before addition to the Assay Plate
- Magnetic stirrer
- Deionized, purified water (e.g. 1.2L of 18 megaohm water)
- Vortexer
- Microplate reader equipped to measure absorbance at 450 nm and software such as Gen5, SoftMax Pro or equivalent capable of performing 4-PL regression.
- Water bath or dry heat block for heat-inactivating virus (e.g. 1 hour at 56°C) potentially present in samples (optional, if desired)

Assay Processing Platforms

The AntiCoV-ID™ IgG ELISA is capable of being performed in manual mode, or alternatively, the assay Test Procedure may be performed on automated ELISA analysis platforms. Customers wishing to perform the AntiCoV-ID™ IgG ELISA on automated platforms are encouraged to reach out to Akston Biosciences technical staff for assistance.

Reagents

Each Akston Biosciences AntiCoV-ID™ IgG ELISA test kit (Part No. 600016) contains reagents for 96 wells, sufficient for 76 samples in singlet or 38 samples in duplicate, 2 controls (Assay Control 1 and Assay Control 2 which contain buffered solutions of a recombinant anti-SARS-CoV-2 antibody), and 1 calibration curve in duplicate. When running multiple assay plates at once, best results may be obtained by pooling identical reagents from identical lots. Expiry dates may be found on the outer packaging of the kit.

Assay Plate	1 plate	96 wells 8-well strips	Ready for use
IgG Calibrator pre-coated strips (with **GOLD** ● markings)			x 2
Recombinant SARS-CoV-2 RBD coated strips			x 10
Assay Control 1 GRAY ●	1 vial	0.3 mL	Ready for use
Assay Control 2 GREEN ●	1 vial	0.3 mL	Ready for use
10X Enzyme Conjugate **BLACK** ●	1 bottle	1.5 mL	Preparation required, see below
Enzyme Conjugate Diluent **BLUE** ●	1 bottle	15 mL	Ready for use
Sample Dilution Buffer YELLOW ○	1 bottle	30 mL	Ready for use
10X Wash Buffer **VIOLET** ●	2 bottles	60 mL	Dilute 100 mL with 900 mL of deionized water to give 1X Wash Buffer
TMB Substrate **WHITE** ○	1 bottle	15 mL	Ready for use
Stop Solution 1% sulfuric acid **RED** ●	1 bottle	15 mL	Ready for use

Specimen Collection and Handling

Serum

With the help of a trained phlebotomist, collect a blood sample via venipuncture in a serum separator tube (SST) or an equivalent tube that allows the blood to clot. Separate the blood from serum by centrifugation according to the blood collection or SST tube manufacturer's instructions. Store samples at 2-8°C for shorter times, or freeze serum and store at -20°C for longer timeframes. Repeated freeze-thaw cycles should be avoided.

Plasma

With the help of a trained phlebotomist, collect a blood sample via venipuncture in a sodium citrate or potassium EDTA collection tube that prevents clotting. Separate the blood from plasma by centrifugation according to the blood collection tube manufacturer's instructions. Store samples at 2-8°C for shorter times, or freeze plasma and store at -20°C for longer timeframes. Repeated freeze-thaw cycles should be avoided.

Heat-inactivated Serum and Heat-inactivated Plasma

In some cases when dealing with potentially infectious samples, heat treatment may lead to the inactivation of pathogens in a biospecimen. Collect serum or plasma as described above and aliquot a small volume into a small microtube with a tight lid. Incubate the sample in a water bath or dry heat block at 56°C for a minimum of 1 hour. Treatment in this manner may help inactivate pathogens in the biospecimen. Heat-inactivation is not a substitute for proper laboratory safety; even with heat-inactivation, use universal precautions and proper personal protective equipment when working with potentially infectious samples. Consult your facility's biosafety officer with questions. For more information on heat-inactivated samples for clinical testing, see page 15.

Preparation of samples

Samples should be diluted from 1:50 to 1:400 or higher as needed in the provided Sample Dilution Buffer. Based on our experience, a 1:100 dilution is optimal for assay performance, and assay validation was performed at a 1:100 dilution level. See the detailed Test Procedure for more details. See page 15 for sample stability and storage.

Kit and Reagent Storage and Stability

Store the AntiCoV-ID™ IgG ELISA test kit at a temperature between 2–8°C, and do not freeze. Unopened, all test kit components are stable until the indicated expiry date. In use stability following the first opening: after opening, the reagents are stable until the indicated expiry date when stored at 2–8°C and protected from contamination, unless stated otherwise.

AntiCoV-ID™ IgG ELISA Test Procedure

Precautions: Human serum/plasma samples should be treated as potentially infectious biological samples and all precautions for blood borne pathogens should be taken, including use of appropriate personal protective equipment such as lab coats, gloves, face shield/eye goggles, and masks. Use of biosafety level 2 cabinets is highly recommended. Additional precautions may include the inactivation of virus by heat treatment at 56°C for 1 hour prior to use.

1. Leave the assay AntiCoV-ID™ IgG ELISA test kit at room temperature for not less than 1 hour before conducting the assay (all reagents must be brought to room temperature prior to the assay).
2. Dilute samples before adding to the Assay Plate wells (see Page 13 - Assay Plate Layout). Dilute each sample in Sample Dilution Buffer (**YELLOW** ○) at a suggested ratio of 1:100. A two-step dilution is suggested as follows:
   - *1:10 dilution: 10 µL serum/plasma + 90 µL Sample Dilution Buffer, mix well*
   - *1:100 dilution: for duplicate well analyses (25 µL of 1:10 dilution + 225 µL Sample Dilution Buffer, mix well); for single well analyses (12 µL of 1:10 dilution + 108 µL Sample Dilution Buffer, mix well).*
3. Open the sealed pouch and unpack the Assay Plate containing the calibrator IgG coated 8-well strips and the spike protein RBD coated 8-well strips. Orient the plate as shown in the Assay Plate Layout on Page 13.
4. Load 100 µL Sample Dilution Buffer into each well of the two calibrator IgG standard strips. The Calibrator IgG standard strips are marked with **GOLD** ● dots, and they are precoated at the following nominal concentrations:

Calibrator Strip Well	Concentration (µg/mL)	Concentration (ng/mL)
A1,A2	2.000	2000
B1,B2	0.800	800
C1,C2	0.320	320
D1,D2	0.128	128
E1,E2	0.0512	51.2
F1,F2	0.0205	20.5
G1,G2	0.00819	8.19
H1,H2	0.00328	3.28

5. Gently mix or vortex Assay Controls. It is recommended to centrifuge Assay Controls 1 and 2 to recover any liquid trapped on the inside of the vial cap.
6. Load 100 µL Assay Control 1 (**GRAY** ●) into wells A3 and A4.
7. Load 100 µL Assay Control 2 (**GREEN** ●) into wells B3 and B4.
8. Add 100 µL of the 1:100 diluted patient samples from Step 2 into each well within the sample area of the plate (see non-shaded area of Assay Plate Layout), and record the sample IDs in the plate layout.
9. Place the Microplate Adhesive Film on top of the plate. Incubate the plate for 1 hour at room temperature without shaking.

10. Dilute the 10X Wash Buffer (VIOLET ●) with deionized water as follows:
    - 100 mL 10X Wash Buffer concentrate + 900 mL deionized or distilled water to give 1X Wash Buffer.
    - NOTE: 2 x 60 mL bottles of 10X Wash Buffer (VIOLET ●) are provided.
11. Dilute the 10X Enzyme Conjugate (BLACK ●) with Enzyme Conjugate Diluent (BLUE ●) as follows, then set aside and keep in the dark:
    - 1.2 mL 10X Enzyme Conjugate + 10.8 mL of Enzyme Conjugate Diluent to give 1X Enzyme Conjugate.
12. Wash the plate five times in a plate washer or manually using a multichannel pipettor as follows:
    - 300 µL wash buffer per well for each wash cycle (shaking or tapping between each wash cycle is recommended). Aspirate all wash buffer after the last wash cycle, and pat dry on an absorbent paper towel.
13. Add 100 µL of diluted 1X Enzyme Conjugate solution per well to all wells. Place the Microplate Adhesive Film on top of the plate. Incubate the plate at room temperature in the dark for 1 hour, without shaking.
14. Wash the plate five times in a plate washer or manually using a multichannel pipettor as follows:
    - 300 µL wash buffer per well for each wash cycle (shaking or tapping between each wash cycle is recommended). Aspirate all wash buffer after the last cycle, and pat dry on an absorbent paper towel.
15. Wash the plate once more using a multichannel pipettor as follows:
    - 300 µL deionized water per well. Aspirate all wash buffer after the last cycle, pat dry on an absorbent paper towel.
16. Add 100 µL of TMB Substrate (WHITE ○) per well to all wells column by column. Incubate the plate in dark for 25 minutes (or until sufficient color development is achieved in the standard strips). A faint to dark blue color will develop.
17. Add 100 µL of Stop Solution (RED ●) to all wells to stop the reaction, in the same order and pace as the TMB Substrate was added in step 16. The blue color will turn to a faint/dark yellow color, with the amount of yellow color being proportional to the level of anti-spike protein RBD antibodies in the samples. Gently tap plate to ensure good mixing of Stop Solution in all wells.
18. Read the plate absorbance at 450 nm wavelength in a microplate reader within 30 minutes. Example OD450 values for standards can be found on page 18.
19. Using the appropriate plate reader software (e.g. SoftMax Pro or Gen 5), create a standard curve using a 4-PL model curve fit to the first two IgG calibrator standard strip concentrations (see Step 4 for IgG calibrator concentrations needed to construct the standard curve) and interpolate

Sample absorbances to obtain anti-spike protein RBD antibody concentrations expressed as human IgG units in µg/mL or ng/mL. The concentrations obtained should be multiplied by the sample dilution factor to obtain the final antibody titer values (e.g. for samples diluted 1:100, the obtained concentration should be multiplied by 100).

20. Results of the assay are valid if the reported Assay Controls meet both of the following criteria:

Validity Check	Value obtained is within +/- 30% of the following values
Assay Control 1 (GRAY ●)	<Value reported on Assay Control 1 vial label>
Assay Control 2 (GREEN ●)	<Value reported on Assay Control 2 vial label>

21. If both of the Assay Control criteria are not met, then the assay results are NOT valid.
22. When finished, dispose of all reagents, microplate, and unused kit materials according to your facility's environmental, health, and safety protocols.
23. Notes:
    - Samples demonstrating very high antibody titers (e.g. reading at or above the AntiCoV-ID™ IgG ELISA ULOQ) may be re-tested with a higher dilution (e.g. 200-fold, 400-fold or higher) to obtain more reliable antibody titer levels.
    - Be careful not to contaminate the TMB Substrate with 1X Enzyme Conjugate solution or Stop Solution.

Limitations of the Procedure

As with all tests, a definitive clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated. Highly lipemic or hemolyzed samples may result in a degradation of the antigen coated on the plate and may result in falsely low assay values, and thereby contribute to higher interassay variation. Time and temperature may exacerbate this assay variation. Keep hemolyzed samples cold or on ice to prevent protein degradation.

Assay Performance Characteristics

The AntiCoV-ID™ IgG ELISA was fully validated under Akston's Quality System using prospective validation comprising both technical and clinical validation sections.

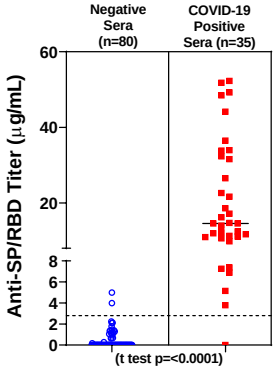
Clinical Performance and Cutoff Value

Akston recommends interpreting a POSITIVE/NEGATIVE cutoff for demonstrating clinically relevant levels of IgG-class antibodies against SARS-CoV-2 spike protein RBD as follows, after back multiplying the sample concentrations by the sample dilution factor to obtain a final antibody titer:

1. Locate the POSITIVE/NEGATIVE CUTOFF VALUE provided on the label affixed to Page 20 (back cover) of this Instructions for Use. Note that this value is only valid for non-heat treated samples.
2. If a sample demonstrates a numeric value LESS than the CUTOFF VALUE, then that sample should be reported as NEGATIVE.
3. If a sample demonstrates a numeric value that is EQUAL OR GREATER than that of the CUTOFF VALUE, then that sample should be reported as POSITIVE, and reported with the absolute numeric value in micrograms per mL ("µg/mL").

Sensitivity, Specificity, and Clinical Agreement

Diagnostic sensitivity and specificity were determined by using 3 separate lots of the AntiCoV-ID™ IgG ELISA to test serum from N=80 COVID-19 negative donors (samples collected prior to November 2019 in the United States and Germany) at a 1:100 dilution according to the Instructions for Use. Of the 80 samples tested, n=78 samples were determined to be antibody negative, resulting in a specificity of 97.5%. Additionally, N=35 serum samples from COVID-19 PCR positive donors, collected at least 2 weeks after symptom onset, were assayed to determine diagnostic sensitivity. N=34 samples gave values above the cutoff, for a sensitivity of 97.1%. Specificity and sensitivity were identical on all three lots of kits tested.



		Clinical Covid-19 Status	
		Positive	Negative
AntiCoV-ID™ IgG ELISA	Positive	34	2
	Negative	1	78

Clinical Performance Summary and Duplicate and Singlet Well Validation

Sensitivity (Positive Percent Agreement; PPA)	True Positives/ (True Positives + False Negatives)	97.1%
Specificity (Negative Percent Agreement; NPA)	True Negatives/ (True Negatives + False Positives)	97.5%
Positive Predictive Value (PPV)	True Positives/ (True Positives + False Positives)	94.4%
Negative Predictive Value (NPV)	True Negatives/ (True Negatives + False Negatives)	98.7%

Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value were analyzed for duplicate well assay format and single well assay format. Duplicate well and single well assay analyses over three assay kit production lots gave the same results and clinical values.

Cross-Reactivity to Non-Coronavirus Antibodies

Serum collected from patients with known IgG titers against the following non-coronaviruses (collected prior to November 2019) were tested on the AntiCoV-ID™ kit at a 1:100 dilution: Mumps (N=8), Measles (N=8), Epstein-Barr virus (EBV; N=8), Cytomegalovirus (CMV; N=8), Varicella zoster virus (VZV; N=8), influenza virus (N=10). None of the samples from any of these donors demonstrated any cross-reactivity on the AntiCoV-ID™ IgG ELISA assay.

ID	Panel	N	AntiCov-ID ELISA	
			Negatives	% Negative
1	Anti-Mumps IgG Antibodies	8	8	100%
2	Anti-Measles IgG Antibodies	8	8	100%
3	Anti-EBV/Anti-Epstein-Barr Nuclear Antigen IgG Antibodies	8	8	100%
4	Anti-CMV IgG Antibodies	8	8	100%
5	Anti-VZV IgG Antibodies	8	8	100%
6	Anti-Influenza IgG Antibodies	10	10	100%

Cross-Reactivity to Non-SARS Coronavirus/Other Anti-Virus Antibodies

An analysis on serum samples collected from N=105 patients with known IgG titers against non-SARS coronaviruses (229E, HKU1, NL63 and OC43) and other viruses was conducted at a Tier 1, US-based university hospital system, and the AntiCoV-ID™ IgG ELISA showed essentially no cross-reactivity to any of the samples (results shown on page 14).

Panel Containing IgG Against	N	Negative	% Negative
Adenovirus E	4	4	100%
Coronavirus 229E	8	8	100%
Coronavirus HKU1	6	6	100%
Coronavirus NL63	9	8	89%
Coronavirus OC43	15	15	100%
Coxsackie/Echovirus	12	12	100%
Influenza A	2	2	100%
Influenza B	6	6	100%
Influenza A, novel H1N1	8	8	100%
Parainfluenzavirus 1	2	2	100%
Parainfluenzavirus 3	2	2	100%
Parainfluenzavirus 4	2	2	100%
Metapneumovirus (A,B)	2	2	100%
Rhinovirus	11	11	100%
Rhinovirus & Coxsackie/Echovirus	14	14	100%
RSVA	2	2	100%
Total	**105**	**104**	

Potential Cross-Reactivity to SARS-CoV

The AntiCoV-ID™ IgG ELISA kit performance has demonstrated very low or zero cross-reactivity to other coronaviruses. However, it is possible that the kit could demonstrate some degree of cross-reactivity to antibodies present in serum or plasma from patients who were exposed to the original SARS-CoV virus ~15 years ago, owing to the fact that the RBD region of SARS-CoV shows a high degree of homology with the RBD region of the SARS-CoV-2 virus. More studies are warranted to experimentally measure the cross reactivity of the AntiCoV-ID IgG ELISA to serum containing antibodies against the original SARS-CoV virus.

Isotype Specificity

Specificity of the AntiCoV-ID™ IgG ELISA test kit for IgG vs. IgM antibodies was demonstrated by spiking monoclonal human IgG and IgM anti-spike protein RBD antibodies, either alone or in combination, into 5 normal serum samples. Samples were then run as usual, with anti-human IgG detection reagent, or using an anti-human IgM detection reagent coupled with an IgM standard curve (a separate positive/negative cutoff was determined for IgM using N=36 normal serum samples).

All spike protein RBD IgM antibody spiked serum samples were negative in the AntiCoV-ID™ IgG ELISA, but positive using the IgM detection reagent, demonstrating that the anti-human IgG-HRP detection antibody (enzyme conjugate) used in the assay kit does not have any cross reactivity to human IgM antibodies. All double (IgG and IgM) spiked serum samples were detected as positives and the IgG titer recovery was >90% for all spiked samples,

demonstrating that the ability of human spike protein RBD IgM antibodies to compete with IgG and produce false negative results is minimal. Additionally, seven COVID-19 positive patient serum samples were assayed using the AntiCoV-ID™ IgG ELISA with and without DTT treatment (to inactivate IgM) to demonstrate that presence of spike protein RBD IgM antibodies does not have the potential to interfere with IgG antibodies and cause false negative results. IgM levels were reduced in all samples after DTT treatment, but measured IgG titers were not significantly affected, and all samples remained IgG "positive" after DTT treatment.

Matrix Equivalency

The AntiCoV-ID™ IgG ELISA test kit is validated for use with human serum, sodium-citrate plasma, and potassium-EDTA plasma. Plasma samples from COVID-19 positive patients (N=7 for sodium-citrate plasma, N=4 for potassium-EDTA plasma) were evaluated in parallel with serum samples from the same patients. Clinical diagnostic agreement was 100%, with all COVID-19 positive plasma samples returning titers above the positive/negative cutoff. Assay values were generally equivalent between plasma and serum samples, with 10 of 11 plasma samples giving 90-120% assay titer agreement.

Matrix Heat-Inactivation Study

Serum and plasma from N=80 negative patients and N=35 COVID-19 positive patients was tested after heat inactivation for 1 hour at 56°C. The samples were run according to the kit Test Procedure with a revised positive/negative cutoff value determined using heat-inactivated, COVID-19 negative serum samples. Assay performance was acceptable and the clinical agreement with non-heat inactivated samples was nearly identical. However, customers wishing to routinely run heat-treated samples are encouraged to re-validate the assay in their laboratories to determine a revised assay cutoff value for heat-inactivated samples.

Heat-Inactivated Samples		**Clinical Covid-19**	
		Positive	**Negative**
AntiCoV-ID™ IgG ELISA	**Positive**	35	2
	Negative	0	78

Sample Stability

Serum samples were validated to be stable for up to 4 hours at room temperature, or up to 24 hours at 4°C. Serum samples were also found to be stable through up to 3x freeze thaw cycles. Sample stability was evaluated by subjecting a normal serum sample spiked with high, mid and low levels of anti-SARS-CoV-2 spike protein specific antibody to the conditions described above, and comparing their assayed titers (at a 1:100 dilution) to freshly thawed, spiked serum samples. Recovery of titer values was found to be within 95-105% of the fresh samples for all tested conditions.

TECHNICAL PERFORMANCE

Limit of Detection/Dynamic Range

The dynamic range of the AntiCoV-ID™ IgG ELISA is 4.69 ng/mL—519 ng/mL, and the assay was shown to demonstrate linearity of dilution across the entire dynamic range. The lower limit of quantitation (LLOQ) for the AntiCoV-ID™ IgG ELISA was determined as 4.69 ng/mL, i.e. sample assay values ≥ 4.69 ng/mL should be considered as reliable. The upper limit of quantitation (ULOQ) was determined as 519 ng/mL (0.519 µg/mL). The end user should consider reanalyzing samples with assay titer values ≥ 519 ng/mL (0.519 µg/mL) with additional dilution to obtain more reliable titer values. Outside of the linear range and above the ULOQ, the assay demonstrates an additional non-linear response upper limit of 950 ng/mL (0.950 µg/mL).

Spike and Recovery

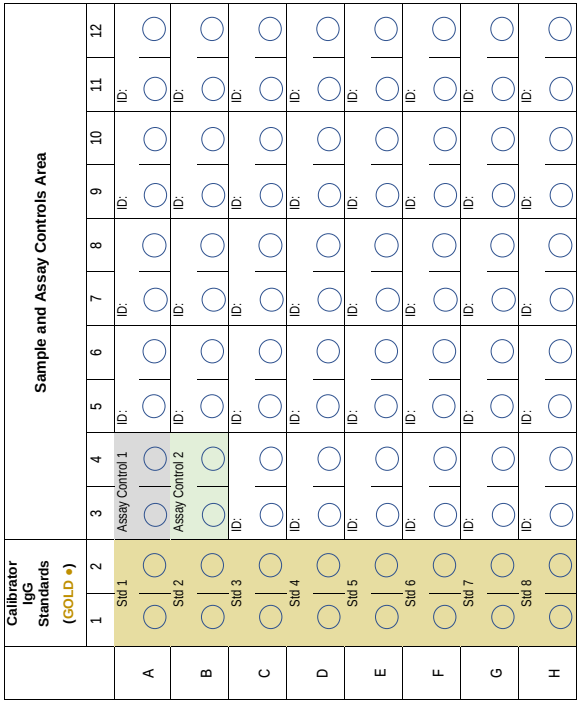
Recovery upon spiking N=12 independent human serum and N=11 human plasma samples taken from COVID-19 negative patients with high, mid, and low levels of anti-SARS-CoV-2 spike protein RBD antibody were found to be 79-118% in all cases.

Accuracy and Precision

The accuracy and precision of the AntiCoV-ID™ IgG ELISA test kit was evaluated using validation samples consisting of QC samples spiked with high, mid and low levels of anti-SARS-CoV-2 spike protein RBD antibody. These samples were tested in 3 sets per run, in 7 assay runs over multiple days by 3 analysts to evaluate accuracy and precision.

		Inter-Assay		**Intra-Assay**	
		Accuracy	**Precision**	**Accuracy**	**Precision**
Validation Sample	**Nominal µg/mL**	***(Avg. % Recovery)***	***%CV***	***(Avg. % Recovery)***	***%CV***
High	0.235	102%	6.7%	95%-116%	1%-4%
Mid	0.173	102%	6.0%	94%-115%	1%-2%
Low	0.078	101%	6.7%	94%-113%	0%-3%

ASSAY PLATE LAYOUT



Calculation of Results

The concentration of anti-SARS-CoV-2 spike protein RBD IgG antibody in a sample is obtained as described in step 19 of the assay Test Procedure. The unknown concentration is obtained by computerized data reduction of the absorbance data for the IgG Calibrator strip wells versus the IgG Calibrator concentrations using a four parameter logistical (4-PL) regression. Reporting of positive/negative status and the reporting of quantitative anti-SARS-CoV-2 spike protein RBD antibody titers is described on page 12, Clinical Performance – Cutoff Value section.

Example Values for Standards

The absorbance values in the table below were collected during 3 independent runs, conducted over multiple days. These values are for reference only, and should not be used for the analysis of data.

Std (Wells)	OD (450 nm)		
	Run1	Run2	Run3
Std1 (A1,A2)	3.172	3.026	2.984
Std2 (B1, B2)	2.321	2.234	2.169
Std3 (C1, C2)	1.332	1.312	1.269
Std4 (D1, D2)	0.654	0.659	0.636
Std5 (E1, E2)	0.297	0.299	0.282
Std6 (F1, F2)	0.154	0.156	0.149
Std7 (G1, G2)	0.090	0.093	0.089
Std8 (H1, H2)	0.071	0.070	0.070

Warranty

The performance of the assay and data provided were obtained following the assay test procedure outlined as indicated and at the recommended sample dilution levels and the product. If the test procedure is performed as described the Product will perform as intended for a period of at least 3 months from the date of manufacture. This Warranty shall not apply to any product that shall have been altered in any way, nor to any product that shall have been used or applied contrary to the printed instructions. NO OTHER WARRANTY EXPRESSED OR IMPLIED, WHETHER OF FITNESS FOR A PARTICULAR PURPOSE OR OF MERCHANTABILITY OR OF ANY OTHER KIND, SHALL EXIST IN RESPECT TO SUCH PRODUCT.

References

Jun Lan, et al. “Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor”, *Nature* 2020.

OVERVIEW OF TEST PROCEDURE
Akston Biosciences AntiCoV-ID™ IgG ELISA

Prepare diluted patient samples at suggested 1:100 dilution in Sample Dilution Buffer (SDB)	1st dilution: 10 µL sample + 90 µL SDB; 2nd dilution: Duplicate sample: (25 µL of 1:10 solution + 225 µL of SDB to achieve 250 µL at 1:100 dilution) OR Singlet sample: (12 µL of 1:10 solution + 108 µL of SDB to achieve 120 µL at 1:100 dilution)
Add Sample Dilution Buffer to Calibrator Strips	100 µL/well
Add Assay Controls 1 and 2, add diluted Samples	100 µL/well
Incubate	1 hour, at room temperature (without shaking)
Wash plate with 1X Wash Buffer	300 µL/well, 5 times
Add 1X Enzyme Conjugate	100 µL/well
Incubate	1 hour, at room temperature, protect from light (without shaking)
Wash plate with 1X Wash Buffer	300 µL/well, 5 times
Wash plate with Deionized Water	300 µL/well, once only
Add TMB Substrate	100 µL/well
Incubate	25 minutes or longer at room temperature, protect from light
Add Stop Solution	100 µL/well
Measure absorbance at 450 nm	Calculate results

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For more information, please visit:

www.anticov-id.com

For technical assistance, please email: techsupport@akstonbio.com

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#**MAS0010/MAS0011-IVDR07**