ARBOR ASSAYS™
Interactive Assay Solutions™

DetectX®

SARS-CoV-2 IgG Antibody
Enzyme Immunoassay Kit

1 Plate Kit	Catalog Number K075-H1R
5 Plate Kit	Catalog Number K075-H5R

Human Specific

For Research Use Only

Sample Types Validated:

Serum

Please read this insert completely prior to using the product.

Not for the diagnosis of SARS-CoV-2 infection. This device has not been FDA cleared or approved. For research use only. Not for use in clinical or diagnostic procedures.

www.ArborAssays.com

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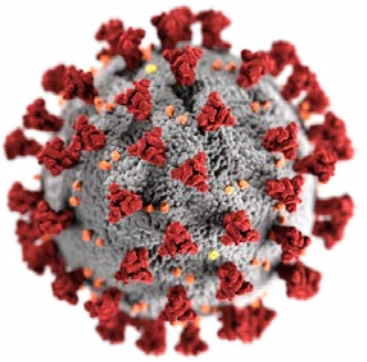
BACKGROUND

COVID-19 is the name for the pandemic disease caused by the SARS-CoV-2 virus. The first case is thought to have occurred in mid-November in Wuhan, the capital of Hubei province, in China. The virus causes a sometimes fatal outcome in humans. The Chinese CDC informed the World Health Organization (WHO) on December 31st 2019. By January 30th 2020 the WHO declared the disease an Emergency of International Concern. On March 11th the WHO declared it a pandemic emphasizing that it had spread rapidly across all continents and almost every country in the world.



The SARS-CoV-2 virus is a positive-sense single stranded RNA virus closely related to other Severe Acute Respiratory Syndrome (SARS) coronaviruses. SARS-CoV-2 has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins. The N protein holds the RNA genome, while the S, E, and M proteins together create the viral envelope. The S protein mediates viral entry into host cells by first binding to the host ACE2 receptor through the receptor-binding domain (RBD) in the S1 subunit and then fusing the viral and host membranes through the S2 subunit. The RBD residues 331 to 524 of the S1 protein elicits the production of antibodies in the host.

Primary detection of SARS-CoV-2 infection is through PCR-based assays from throat and nasal swabs. These assays detect the RNA of the virus. Serological assays allow the study of the immune response to SARS-CoV-2 in a qualitative and quantitative manner. IgG and IgM assays are needed to determine the precise rate of infection in an affected area, which is an essential variable to accurately determine the infection fatality rate. Serological assays will allow for the identification of individuals who mounted strong antibody responses and who could serve as donors for the generation of serum antibody-based therapeutics. Assays will also permit health authorities to determine who may be immune and who is not. This information may be very useful for deploying healthcare workers in a strategic manner to limit the risk of exposure and inadvertent spread of the virus. It could also allow proportions of the population that have already acquired immunity to go back to ‘normal life’.

ASSAY PRINCIPLE & INTENDED USE

The DetectX® SARS-CoV-2 IgG ELISA kit is designed to qualitatively measure SARS-CoV-2 viral reactive IgG species in human serum.

Please read the complete kit insert before performing this assay. Ready to use human negative and positive controls are provided to generate negative (low) and positive (high) optical densities (OD) for the assay. The OD readings for these two controls needs to fall within acceptable limits for the assay to be considered valid. A clear microtiter plate coated with SARS-CoV-2 recombinant S and N proteins designed to capture reactive antibodies in blood from people infected with SARS-CoV-2 is provided. Controls or diluted samples are pipetted into the wells and the plate is shaken at room temperature for 30 minutes. After the incubation the plate is washed and a goat antibody to human IgG conjugated to peroxidase conjugate is added to the wells and the plate is shaken at room temperature for 30 minutes. The conjugate will bind to any human sample IgG captured by the recombinant proteins on the plate. The plate is washed and substrate is added to all the wells. The substrate reacts with the bound goat antibody to human IgG-peroxidase conjugate, and after a short incubation, the reaction is stopped. The intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The presence of SARS-CoV-2 IgG in the samples is assessed by comparing with the positive and negative control ODs and the published cutoff ODs on page 11.

WARNINGS:

For research use only.

Negative results do not rule out SARS-CoV-2 infection.

Results from antibody testing should not be used for clinical or diagnostic procedures.

Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains such as HKU1, NL63, OC43 or 229E.

This assay is not for the screening of donated blood.

RELATED PRODUCTS

Kits	Catalog No.
SARS-CoV-2 IgG ELISA Kit (384-Well Plate) - COMING SOON	K075-H1DR
SARS-CoV-2 IgM ELISA Kits - COMING SOON	K074-H1/H5R
SARS-CoV-2 IgM ELISA Kit (384-Well Plate) - COMING SOON	K074-H1DR
2',3'-Cyclic GAMP ELISA Kits	K067-H1/H5
2',3'-Cyclic GAMP ELISA Kit (384-Well Plate)	K067-H1D
3',3'-Cyclic GAMP ELISA Kits	K073-H1/H5
Prostaglandin E_2 (PGE_2) Multi-Format ELISA Kits	K051-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates
A clear plastic microtiter plate(s) coated with SARS-CoV-2 recombinant proteins.

Kit K075-H1 **or** -H5	1 **or** 5 Each	Catalog Number C275-1EA

SARS-CoV-2 IgG Positive Control
Heat-inactivated human serum diluted in Assay Buffer with a positive level of SARS-CoV-2 reactive IgG.

Kit K075-H1 **or** -H5	200 µL **or** 1 mL	Catalog Number C281-200UL **or** -1ML

WARNING: This material contains human serum and should be considered a biohazard.

SARS-CoV-2 IgG Negative Control
Heat-inactivated human serum diluted in Assay Buffer with a negative level of SARS-CoV-2 reactive IgG.

Kit K075-H1 **or** -H5	200 µL **or** 1 mL	Catalog Number C282-200UL **or** -1ML

WARNING: This material contains human serum and should be considered a biohazard.

Assay Buffer Concentrate
A 2X concentrate that must be diluted with deionized or distilled water.

Kit K075-H1 **or** -H5	28 mL **or** 2 Each, 60 mL	Catalog Number X148-28ML **or** -60ML

DetectX® Goat anti-human IgG Peroxidase Conjugate
A peroxidase conjugate in a special stabilizing solution.

Kit K075-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number C283-5ML **or** -25ML

Wash Buffer Concentrate
A 20X concentrate that must be diluted with deionized or distilled water.

Kit K075-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K075-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

Stop Solution
A 1M solution of hydrochloric acid. **CAUSTIC**.

Kit K075-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K075-H1 **or** -H5	2 **or** 10 Each	Catalog Number X002-1EA

STORAGE INSTRUCTIONS

The unopened kit must be stored at -20°C. Once opened, the kit can be stored at 4°C up to the expiration date on the kit label, except for the Assay Buffer Concentrate, Positive Control, and Negative Control. These must be stored at -20°C.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Repeater pipet, such as Eppendorf repeater and disposable tips to accurately dispense 50 µL and 100 µL.

A microplate shaker capable of maintaining shaking at approximately 500 RPM.

Colorimetric 96-well microplate reader capable of reading optical density at 450 nm.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The Positive and Negative Controls supplied with this kit are derived from human blood and should be treated as potentially infectious. They have been tested negative for Hepatitis B and C, HIV and other infectious diseases. Appropriate precautions should be taken.

The protein coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers wash buffers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free and ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on page 8.

After running the assay, the used assay wells, pipette tips, diluted samples, etc are biohazardouswaste and should be treated appropriately and disposed of according to local regulations.

Safety Data Sheet (SDS) is available for this product. Review before performing this assay.

SAMPLE TYPES

We recommend studying the Centers for Disease Control guidelines for handling potential biological hazards that may contain COVID-19 infective material. Please see: https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html for more information.

This assay has been tested and validated for human serum samples.

Blood collected should be allowed to clot at room temperature for 30 to 60 minutes and the centrifuged to collect serum. The serum can be removed from the clot and stored at 4°C for short periods of time (≤ 4 days) or at ≤ -70°C for long term storage. Avoid multiple freeze-thaw cycles to protext sample integrity. Samples containing visible particulate should be centrifuged prior to use.

If sera is to be shipped, package to ensure samples remain frozen in transit and mark as biohazards.

SAMPLE PREPARATION

We recommend heat treating serum samples to 56°C for 1 hour to inactivate any viral pathogens that may be present prior to analysis.

Samples are sometimes heated to inactivate potential infectious virus infections. We evaluated the effect of 56°C for 1 hour on sample values. PCR positive sample values increased 4.4% with heat treatment. PCR negative samples OD values increased approximately 23.3% with heat treatment.

Samples must be diluted in Assay Buffer prior to running the assay. An initial dilution of 1:100 is made by adding 1 part of sample to 99 parts of Assay Buffer. A minimum volume of 100 µL of diluted sample is required for duplicate determinations. We recommend adding 5 µL of serum sample to 495 µL of diluted Assay Buffer.

Use all samples within 2 hours.

NOTE: Positive and Negative Control should be assayed in duplicate and run on every plate each time an assay is performed.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30-60 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Wash Buffer
Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted, this is stable at room temperature for 3 months.

Assay Buffer
Dilute Assay Buffer Concentrate 1:2 by adding one part of the concentrate to one part of deionized water. Once diluted, this should be aliquoted and stored at -20°C up to the expiration date on the kit label.

Negative Control
The Negative Control is prediluted in Assay Buffer and ready to use. **CAUTION: The Negative Control sample is human serum based.**

Positive Control
The Positive Control is prediluted in Assay Buffer and ready to use. **CAUTION: The Positive Control sample is human serum based.**

ASSAY CONSIDERATIONS

1. Positive and Negative Control should be assayed in duplicate and run on every plate each time an assay is performed.
2. Room temperature incubation is at 23–25°C. Temperature will affect assay signal.
3. Incubation steps requiring shaking must be at approximately 500 RPM.

   Signal will be approximately 25% lower without shaking.

   Signal will be approximately 15% lower with slower speed and 15% higher with increased speed.
4. Use of a plate sealer is optional. If a plate sealer is used, the signal for positive samples will be increased by approximately 20%.

ASSAY PROTOCOL

We recommend that all controls and samples be run in duplicate to allow the end user to reliably determine the positive or negative level of the samples.

1. Use the plate layout sheet on the back page to aid in proper sample and control identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet 50 µL of the Positive Control provided into duplicate wells.
3. Pipet 50 µL of the Negative Control provided into duplicate wells.
4. Pipet 50 µL of samples into wells in the plate.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer (optional) and shake at room temperature for 30 minutes.
6. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer. Tap the plate dry on clean absorbent towels. **The aspirated wash solution should be treated as a biohazard and treated prior to disposal.**
7. Add 50 µL of the DetectX® Goat anti-human IgG Conjugate to each well using a repeater pipet.
8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer (optional) and shake at room temperature for 30 minutes.
9. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer. Tap the plate dry on clean absorbent towels.
10. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
11. Incubate the plate at room temperature for 30 minutes without shaking.
12. Add 50 µL of the Stop Solution to each well using a repeater pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

CALCULATION OF RESULTS

Average the duplicate OD readings for each control and sample.

Verify positive and negative control readings fall within acceptable ranges to assess sample readings against cut-off ranges. If they do not, the assay must be repeated. If they do, refer to Sample Interpretation below to assess sample readings.

Control	Acceptable OD Range
Positive	1.0 – 1.4
Negative	0.12 – 0.30

TYPICAL DATA

Sample	Mean OD	Result
Positive Control	1.161	–
Negative Control	0.213	–
Sample 1	0.174	Negative
Sample 2	> 4.00	Positive
Sample 3	1.307	Positive
Sample 4	0.433	Retest

Always run the Positive and Negative controls provided for calculation of results. Do not use this data.

Sample Interpretation

Result	Cut-Off Ranges	Explanation
Negative	≤ 0.329 OD	3.5 SD above mean negative sample.
Retest	0.33 – 0.499 OD	Sample should be repeated in at least duplicate to verify sample status. If repeat falls within “retest,” it is recommended another sample is collected from the same patient at a later date.
Positive	≥ 0.5 OD – Max OD	7.0 SD above mean negative sample. If sample at 1:100 gives OD reading as overflow, it is a positive result.

VALIDATION DATA

Negative and Positive Cutoff Optical Density Determination

Multiple (40) samples negative for SARS-CoV-2, collected before 2019, were tested in the assay. The mean OD obtained was 0.157 OD with a standard deviation of 0.049 OD. The negative cut-off value was set at 3.5 SDs above the mean, with a value of 0.329 OD.

Multiple (45) PCR-positive samples from patients confirmed for SARS-CoV-2 were tested in the assay and resulted in a range of 0.454 to > 4.000 OD at 450 nm. PCR-positive samples reading higher than 4.0 OD will read as overflow. Plate can be read at 405 nm to get a semiquantitative measurement. The 405 nm data cannot be used for sample interpretation.

PERFORMANCE CHARACTERISTICS

Clinical Agreement

Sensitivity, or Positive Percent Agreement (PPA), is the number of true positive (PCR-Confirmed) samples that read positive in the assay.

Specificity, or Negative Percent Agreement (NPA), is the number of true negative (PCR-Confirmed or collected before 2019) samples that read negative in the assay.

Confirmed Samples	Number of Samples Tested	Number of Samples with Agreement	% Agreement
Positive	48	48	100% PPA
Negative	91	88	96.7% NPA

Cross Reactivity

Above 95% specificity of negative samples (91) confirms minimal cross reactivity to other viruses.

Intra-Assay Precision

The positive and negative controls were run in replicates of 20 in an assay. The mean and precision of the signal were:

Control	Mean OD	% CV
Positive	1.229	4.1
Negative	0.180	4.6

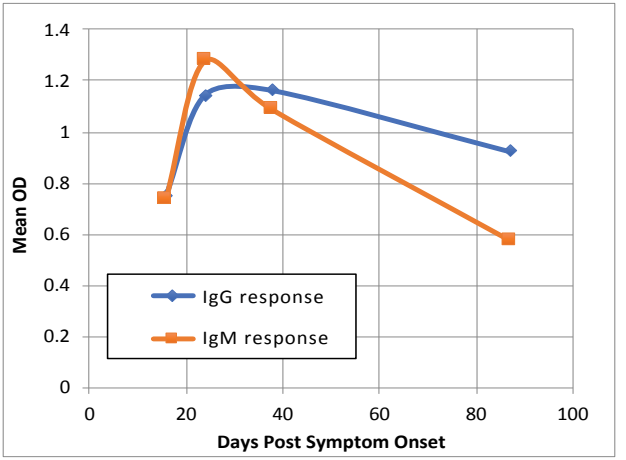
Inter-Assay Precision

The positive and negative controls were run in duplicate in ___ assays run over multiple days by three operators. The mean and precision of the signal were:

Control	Mean OD	% CV
Positive	1.203	7.9
Negative	0.207	20.6

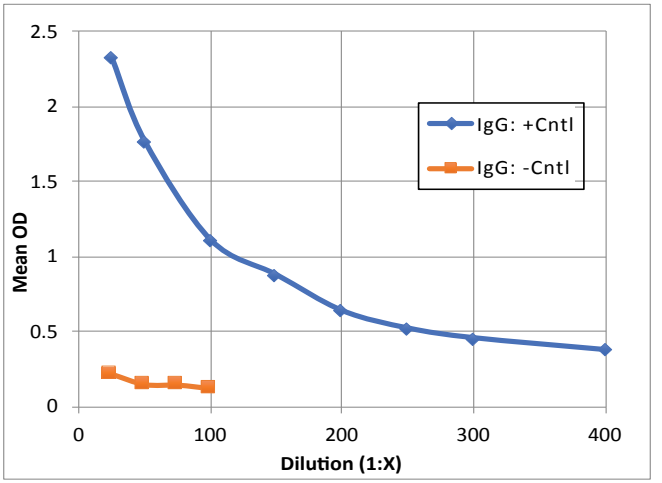
TIME COURSE OF Ig RESPONSE

One PCR positive SARS-CoV-2 patient donated serum on a weekly basis and the IgG and IgM response was monitored through this time period. The response below was observed:



SAMPLE DILUTION RESPONSE

We recommend a 1:100 dilution of serum into diluted Assay Buffer. Samples can be diluted outside of this recommendation and the following graph show the response of the assay to different dilutions.



LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with ELISA kits for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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