SCoV-2 Detect™ Neutralizing Ab ELISA

Instructions for Use

For Emergency Use Authorization (EUA) Only

For *In Vitro* Diagnostic (IVD) Use Only
For Prescription Use Only

INTENDED USE

The SCoV-2 *Detect*™ Neutralizing Ab ELISA is an enzyme-linked immunosorbent assay intended for the qualitative direct detection of total neutralizing antibodies to SARS-CoV-2 in human serum and plasma (dipotassium EDTA, lithium heparin, and sodium citrate). The SCoV-2 *Detect*™ Neutralizing Ab 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 neutralizing antibodies confers protective immunity. The SCoV-2 *Detect*™ Neutralizing Ab ELISA should not be used to diagnose or exclude acute SARS-CoV-2 infection.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.

Results are for the detection of SARS CoV-2 total neutralizing antibodies. Antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time neutralizing 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 SCoV-2 *Detect*™ Neutralizing Ab ELISA 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 SCoV-2 *Detect*™ Neutralizing Ab ELISA may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The SCoV-2 *Detect*™ Neutralizing Ab ELISA is only for use under the Food and Drug Administration Emergency Use Authorization (EUA).

SUMMARY AND EXPLANATION OF THE TEST

The novel coronavirus, SARS-CoV-2 (the causative agent of COVID-19), has been responsible for the pandemic of pneumonia-like symptoms and associated deaths from late 2019 and into 2021. The detection of the initial outbreak in the Hubei Province of China and the subsequent need for an effective diagnosis were quickly described (Li et al., 2020; Wu et al., 2020; Zhou et al., 2020).

It has been reported that RT-PCR-confirmed SARS-CoV-2 positive patients may seroconvert and develop antibodies against SARS-CoV-2 antigens anywhere from 6-21 days after the onset of clinical symptoms (Okba et al., 2020). The specific and reliable detection of human antibodies capable of neutralizing SARS-CoV-2 remains a key method to monitor infections, for effective contact tracing, and for serosurveillance (Okba et al., 2020).

The SCoV-2 *Detect*™ Neutralizing Ab ELISA is a qualitative immunoassay for the detection of antibodies inhibiting the interaction between the human angiotensin converting enzyme 2 (ACE2) receptor and SARS-CoV-2 Spike (S) protein.

PRINCIPLE OF THE TEST

The SCoV-2 *Detect*™ Neutralizing Ab ELISA is a qualitative competitive inhibition ELISA that detects antibodies to SARS-CoV-2 and that prevent binding of the virus to the human ACE2 receptor. Serum or plasma specimens are first diluted and pre-incubated with proprietary monoclonal antibody (mAb) premixed with recombinant SARS-CoV-2 S protein. Then the mixture is transferred to ACE2 receptor protein-coated wells. After incubation and washing, the S protein-mAb immune complex remains bound on the plate surface unless the interaction was inhibited by neutralizing antibodies (NAbs) in the specimen. The protein-protein interaction is designed

to mimic the virus-host interaction. Secondary antibody conjugated to horseradish peroxidase (HRP) targeting the mAb is then added to each well. After incubation, the ELISA wells are washed before a tetramethylbenzidine (TMB) substrate is added. An acidic solution is finally used to stop the reaction and the degree of enzymatic turnover of the substrate is determined by measuring optical density (OD) at 450 nanometers.

Positive and Negative Controls are provided to ensure the integrity of the assay and to determine the assay-specific threshold. Up to 92 specimens may be evaluated with each kit (as the controls are run in duplicate). The entire procedure takes approximately 3 hours from preparing the Sample Dilution Buffer, or approximately 1 hour and 40 minutes once sample dilution is started.

KIT CONTENTS

Warning: Do not use any reagents where damage to the packaging has occurred.

The kit contains the following reagents:

1. **COATED MICROTITER STRIPS FOR SCOV-2 NAB ELISA:** Strip holder in a resealable foil pouch, containing 96 polystyrene microtiter wells coated with receptor protein in each well. Stable at 2-8°C until the expiration date.
2. **SCOV-2 NAB NEGATIVE CONTROL:** One vial, 150 µL. Negative Control sample. The Negative Control will aid in monitoring the integrity of the kit and in interpreting sample results. Stable at 2-8°C until the expiration date.
3. **SCOV-2 NAB POSITIVE CONTROL:** One vial, 150 µL. Positive Control contains neutralizing antibodies and will aid in monitoring the integrity of the kit. Stable at 2-8°C until the expiration date.
4. **100X NEUTRALIZATION MIX FOR SCOV-2 NAB:** One vial, 300 µL. This contains recombinant Spike antigen pre-mixed with proprietary secondary antibody. The 100X Neutralization Mix is added to the Neutralization Dilution Buffer prior to its use. Stable at 2-8°C until the expiration date.
5. **NEUTRALIZATION DILUTION BUFFER FOR SCOV-2 NAB:** One bottle, 25 mL. Tris-HCl buffered solution (pH 7.2-7.6) with Tween 20, preservative and additives. The Neutralization Dilution Buffer will be used to dilute 100X Neutralization Mix prior to mixing with samples and controls. Stable at 2-8°C until the expiration date.
6. **100X CONJUGATE FOR SCOV-2 NAB:** One vial, 100 µL, containing horseradish peroxidase-labeled antibody in a Tris-based buffer with preservative. Stable at 2-8°C until the expiration date.
7. **CONJUGATE DILUENT FOR SCOV-2:** One bottle, 9 mL, is to be used to dilute the 100X conjugate before adding to the ELISA plate. Stable at 2-8°C until the expiration date.
8. **10X WASH BUFFER:** One bottle, 120 mL. 10X concentrated phosphate buffered saline with Tween 20 (pH 6.8-7.0). Stable at 2-8°C until the expiration date.
9. **LIQUID TMB SUBSTRATE:** One bottle, 12 mL, ready to use. Contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide in a citric acid-citrate buffer (pH 3.3-3.8). Stable at 2-8°C until the expiration date. Note: The substrate should always be stored in the light-protected bottle provided.
10. **STOP SOLUTION:** One bottle, 6 mL, ready to use. 1N Sulfuric Acid. Used to stop the reaction. Stable at 2-8°C until the expiration date.

    Warning: Strong acid. Wear protective gloves, mask and safety glasses. Dispose all materials according to all applicable safety rules and regulations.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- ELISA spectrophotometer capable of absorbance measurement at 450 nm
- Biological or high-grade water
- Appropriately sized beakers and stir bars
- Vacuum pump
- Automatic plate washer
- 37°C incubator without CO_2 supply
- 1-10 µL single-channel pipettors, 50-200 µL single- and multichannel pipettors
- Polypropylene tubes or 96 well dilution plates
- Parafilm or plastic plate cover
- Timer
- Vortex

WARNINGS AND PRECAUTIONS

- For use Under Emergency Use Authorization only.
- For *in vitro* diagnostic use only.
- For prescription use only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for detecting the presence of neutralizing antibodies to SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities
- Follow standard precautions. All specimens and controls should be considered potentially infectious and handled in accordance with good laboratory procedure.

SAFETY PRECAUTIONS

- Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke, or apply cosmetics in the laboratory where immunodiagnostic materials are being handled.
- Do not pipette by mouth.

TECHNICAL PRECAUTIONS

- This test must be performed on human serum or plasma only. The use of whole blood or other specimen matrices has not been validated.
- Do not mix various lots of any kit component within an individual assay.
- All reagents must be equilibrated to room temperature (15-25°C) before commencing the assay. The assay will be affected by temperature changes.
- Samples should not be frozen and thawed more than once.
- Dispense reagents directly from bottles using clean pipette tips. Transferring reagents may result in contamination.
- Unused microtiter wells must be resealed immediately in the resealable foil pouch with the desiccant provided. Failure to do so may cause erroneous results with those unused microwells.
- Do not use any component beyond the expiration date shown on its label.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
- Some reagents may form a slight precipitate, mix gently before use. **Do not vortex the 100X Neutralization Mix for SCoV-2 NAb.**
- Incomplete washing will adversely affect the outcome and assay performance.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stop solution into the wells in the same order and speed used to add the TMB solution.
- Avoid microbial contamination of reagents.
- Avoid contamination of the TMB Substrate Solution with the Conjugate Solution. The TMB Substrate Solution should be clear in color; a blue color change prior to use may indicate contamination has occurred.
- Use a clean disposable pipette tip for each reagent, standard, control or specimen.
- Cover working area with disposable absorbent paper.

CHEMICAL HAZARD

Safety Data Sheets (SDSs) are available for all components of this kit. Review all appropriate SDSs before performing this assay and don required Personal Protective Equipment (PPE) as noted. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable SDS for appropriate treatment.

SPECIMEN COLLECTION AND PREPARATION

- Only human serum or plasma (with dipotassium EDTA, lithium heparin, and sodium citrate) should be used for this assay, and the usual precautions for venipuncture should be observed. To obtain serum, blood obtained by venipuncture without anticoagulant should be allowed to clot at room temperature (20-25°C) for 30 to 60 minutes and then centrifuged according to the Clinical and Laboratory Standards Institute (CLSI Approved Guideline – Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; GP44).
- Testing should be performed as soon as possible after collection. Do not leave sera or plasma at room temperature for prolonged periods. Separated serum or plasma should remain at 20-25°C for no longer than 8 hours. If assays are not completed within 8 hours, samples should be refrigerated at 2-8°C. If assays are not completed within 48 hours, or the separated serum or plasma is to be stored beyond 48 hours, serum or plasma should be frozen at or below -20°C.
- Samples should not be frozen and thawed more than once. Frost-free freezers are not suitable for sample storage.
  Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.
- If sera or plasma are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.
- Do not use sera or plasma if any indication of microbial growth is observed.

TEST PROCEDURE

CAUTION: *The test procedure must be strictly followed. Any deviations from the procedure may produce erroneous results. Bring all reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. NOTE: For long-term storage, serum and plasma samples should not be frozen and thawed more than once. Sera and plasma should be further divided into small aliquots and stored at -20°C or below.*

This assay is intended to be performed manually. Plate washing must be performed using a properly calibrated automated plate washer. This kit has not been optimized by InBios for use with a specific automated ELISA processing system. Use with an automated ELISA processing system will require proper validation.

Preparation of Reagents:

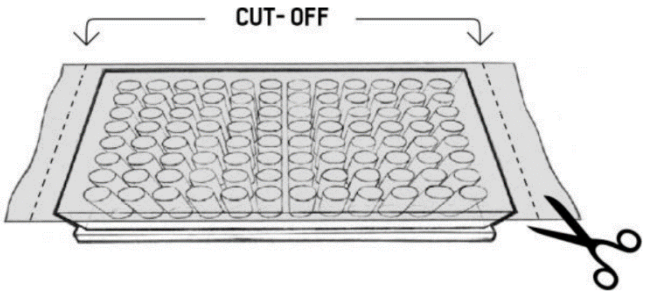
- Preparation of Sample Dilution Buffer
  Dilute 250 µL of the 100X Neutralization Mix for SCoV-2 NAb into the 25 mL bottle of Neutralization Dilution Buffer for SCoV-2 NAb, and gently invert bottle several times. **Do not vortex.** Allow this bottle to equilibrate at room temperature for at least 1 hour before proceeding. Once mixed, this bottle should be used fresh and not stored for further use. .Alternatively, use a clean pipette to remove the required volume of Neutralization Dilution Buffer and add the necessary volume of 100X Neutralization Mix for SCoV-2 NAb into a clean polypropylene test tube in order to maintain the 1:100 ratio.
- Preparation of 1X Wash Buffer
  Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X Wash Buffer solution, mix 120 mL 10X Wash Buffer with 1080 mL distilled (or deionized) water. Mix thoroughly to ensure that any precipitate is dissolved and that the solution is uniform. Once diluted to 1X, the solution can be stored at room temperature for up to 6 months. Properly label the 1X Wash Buffer solution and carefully note the expiration date on the label. Check for contamination prior to use. Discard if contamination is suspected.
- Microtiter Strip Wells
  Select the number of coated wells required for the assay. The remaining unused wells should be repackaged immediately with the supplied desiccant and stored at 2-8°C until ready to use or expiration.
- Preparation of Conjugate Solution
  Add 90 µL of 100X Conjugate for SCoV-2 NAb directly to the 9 mL bottle of Conjugate Diluent for SCoV-2 NAb (1 part : 100 parts). Alternatively, use a clean pipette to remove the required volume of Conjugate Diluent and add the necessary volume of 100X Conjugate for SCoV-2 ELISA into a clean polypropylene test tube in order to maintain the 1:100 ratio. Mix by inverting the solution several times. This conjugate solution

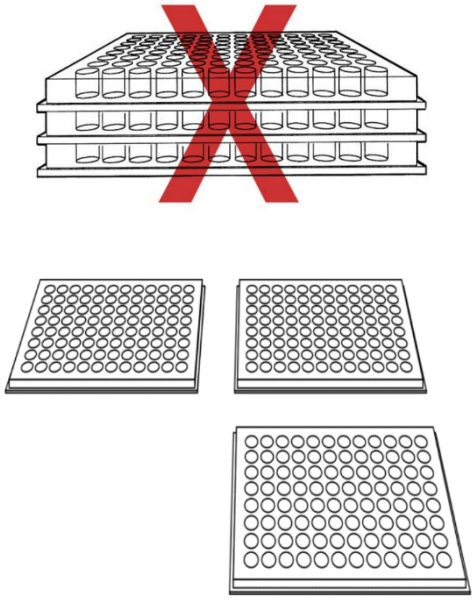
should be prepared immediately prior to running the assay and discarded immediately after use.

Assay Procedure:

1. Positive and negative controls should be assayed in duplicate, and run on every plate, each time an assay is performed. Test unknown serum and plasma samples in singlicate or duplicate. Up to ninety-two test specimens can be tested in singlicate with an entire plate. Immediately place any unused ELISA plate wells back into the original foil packaging with the provided desiccant, properly seal and store at 2-8°C.
2. Dilute each control and each test specimen 1:20 by adding 8 µL of sample to 152 µL of **equilibrated** Sample Dilution Buffer for SCoV-2 NAb. Dilute samples into a dedicated sample dilution block or appropriately sized tubes. Mix samples well by pipetting. Cover the top of the plate with parafilm (or a plastic plate cover) and remove any excess parafilm from the edges of the plate. Incubate these samples at 37°C for 30 minutes.
3. Mix samples again via pipetting. Add 50 µL of the 1:20 diluted controls and test specimens onto the appropriate locations in the SCoV-2 Antigen Coated Microtiter Strip plate (ELISA plate). Note and record the locations of all controls and test samples in the ELISA plate wells.
4. Cover the top of the plate with parafilm (or a plastic plate cover) and remove any excess parafilm from the edges of the plate.

   Note: *This is to make sure the temperature distribution is evenly spread across all wells from bottom and sides; any extra parafilm can be cut off once the top is sealed to block evaporation.*





CORRECT METHOD

Note: *Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO_2 or other gases. Do not place plates in contact with any wet substances such as wet paper towels etc.*

5. Incubate the plate(s) at 37°C for 30 minutes in an incubator.
6. After the incubation, wash the plate 6 times with an automatic plate washer using 1X Wash Buffer. Use 300 µL per well in each wash cycle.
7. Add 50 µL per well of the freshly prepared Conjugate Solution (made from 100X Conjugate diluted into Conjugate Diluent) into all wells using a multi-channel pipettor. Discard the remaining Conjugate Solution.
8. Cover the plate with parafilm or a plastic plate cover and incubate the plate(s) at 37°C for 30 minutes in an incubator.
9. After the incubation, wash the plate 6 times with the automatic plate washer using 1X Wash Buffer. Use 300 µL per well in each wash cycle.
10. Add 75 µL per well of Liquid TMB substrate into all wells using a multi-channel pipettor.
11. Incubate the plate uncovered at room temperature **in the dark**, for 10 minutes.
12. Add 50 µL per well of Stop Solution into all appropriate wells using a multi-channel pipetter. Make sure to add the Stop Solution in the same order and at approximately the same speed at which the TMB was applied. (Note: As the TMB substrate produces an enzymatic reaction with the HRP-conjugate, it is critical this incubation time point is followed as closely as possible). Let the plate stand, uncovered at room temperature, for 1 minute.

13. Read the optical density at 450 nm (OD_{450}) with a microplate reader. DO NOT SUBTRACT OR NORMALIZE ANY BLANK VALUES OR WELLS.
14. Record the raw OD_{450} and evaluate the sample status as indicated in the Quality Control and Interpretation of Results sections.

QUALITY CONTROL

Each kit contains positive and negative controls. These controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay limit of detection. The test is invalid if the control sample values do not fall in the pre-established OD value ranges. If the test is invalid, the results cannot be used, and the testing must be repeated. Quality Control (QC) requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to CLSI C24 and 42 CFR 493.1256 for guidance on appropriate QC practices. The results below are given strictly for guidance purposes only and applicable for spectrophotometric readings only.

In order to establish the test is valid, first calculate the mean (average) negative and positive control raw OD_{450} values as shown in the following examples.

Example 1: SCoV-2 Positive Control

	OD_{450}
Replicate 1	**0.527**
Replicate 2	**0.548**
Sum	**1.075**

Average Positive Control = 1.075 ÷ 2 = 0.5375

Example 2: SCoV-2 Negative Control

	OD_{450}
Replicate 1	**2.675**
Replicate 2	**2.824**
Sum	**5.499**

Average Negative Control = 5.499 ÷ 2 = 2.7495

Finally, verify that the quality control requirements, listed in the table below, are fulfilled.

Quality Control Requirements

Control	Requirement
Positive Control	OD < 1.2
Negative Control	OD > 1.5

The results on the table above must be obtained for the assay to be considered valid. Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated. If repeated invalid tests are reported, contact the manufacturer.

INTERPRETATION OF RESULTS

The cut-off value was determined by screening a large number (277) of normal human serum (NHS) samples that were collected prior to the COVID-19 outbreak (~November, 2019). The cut-off selection was performed by estimating the mean of the negative specimens plus three (3) standard deviations.

Calculate Signal Inhibition (SI %): The test result of the unknown sample is determined by calculating Signal Inhibition. Signal Inhibition can be determined from the OD values of the sample and the Negative Control:

Signal Inhibition (%) = (1 – [Sample OD ÷ Negative Control OD]) * 100%

If unknown samples were tested in duplicate, then calculate the average optical density (OD_{450}) to input as the Sample OD.

Example 3: Calculate the SI % for a Sample

Sample ID	Raw OD_{450}
Unknown Sample #1	1.321
Negative Control	2.985

SI % = (1 – [Sample OD ÷ Negative Control OD]) * 100%

SI % = (1 – [1.321 ÷ 2.985]) * 100%

= 55.7%

SI % *	Results	Interpretation
> 25%	Positive	Neutralizing antibodies for SARS-CoV-2 are detected.
15-25%	Retest	Retest sample in duplicate. If retested Signal Inhibition (%) is ≥ 20%, then neutralizing antibodies for SARS-CoV-2 are detected. If retested SI % is <20%, then neutralizing antibodies for SARS-CoV-2 are not detected.
< 15%	Negative	Neutralizing antibodies for SARS-CoV-2 are not detected.

* Signal Inhibition Results should not be reported to the end user. The clinical applicability of detection or correlation with neutralizing activity for antibodies to SARS-CoV-2 at ≥20% SI is currently unknown and results cannot be interpreted as an indication of degree of immunity or protection from infection. Because SARS-CoV-2 neutralizing antibody assays are not standardized, and the performance characteristics of each SARS-CoV-2 neutralizing antibody test is uniquely established, results from different SARS-CoV-2 neutralizing antibody assays cannot be compared.

LIMITATIONS

- This test is designed for qualitative detection of SARS-CoV-2 neutralizing antibodies.
- To be used only under the conditions of the FDA Emergency Use Authorization.
- Use of the SCoV-2 *Detect*™ Neutralizing Ab ELISA is limited to laboratory personnel who have been trained. Not for home use.
- Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
- The assay performance characteristics have not been established for visual result determination.
- The assay has not been evaluated with fingerstick specimens. This test is not authorized for use with fingerstick whole blood.
- Results from antibody testing should not be used to diagnose or exclude acute COVID-19 infection or to inform infection status.
- 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.
- A negative result for an individual subject indicates absence of detectable anti-SARS-CoV-2 neutralizing antibodies. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. The sensitivity of this assay early after infection is unknown.
- A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limit of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected. Direct testing with a molecular diagnostic should be performed to evaluate for acute SARS-CoV-2 infection in symptomatic individuals.
- It is unknown at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.
- False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
- False positive results due to cross-reactivity with Rheumatoid Factor (RF) can occur.
- Not for the screening of donated blood.
- Samples that are hemolyzed should be avoided for analysis with this assay.
- Results from immunosuppressed patients must be interpreted with caution.
- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.
- The performance of this device has not been established in individuals that have received a COVID-19 vaccine. The clinical significance of a positive or negative antibody result following COVID-19 vaccination has not been established, and the result from this assay should not be interpreted as an indication or degree of protection from infection after vaccination.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected in the US from March 2020 to March 2021. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The SCoV-2 *Detect*™ Neutralizing Ab ELISA Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, 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/in-vitro-diagnostics-euas

Authorized laboratories using the SCoV-2 *Detect*™ Neutralizing Ab ELISA must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

- Authorized laboratories[a] using the SCoV-2 *Detect*™ Neutralizing Ab ELISA must 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.
- Authorized laboratories using the SCoV-2 *Detect*™ Neutralizing Ab ELISA must use the product as outlined in the authorized labeling. 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.
- Authorized laboratories that receive the SCoV-2 *Detect*™ Neutralizing Ab ELISA must notify the relevant public health authorities of their intent to run the assay prior to initiating testing.
- Authorized laboratories using the SCoV-2 *Detect*™ Neutralizing Ab ELISA must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of the SCoV-2 *Detect*™ Neutralizing Ab ELISA and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and InBios Technical Support (https://inbios.com/technical-support/) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the product of which they become aware.
- All laboratory personnel using the SCoV-2 *Detect*™ Neutralizing Ab ELISA must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the SCoV-2 *Detect*™ Neutralizing Ab ELISA 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 the SCoV-2 *Detect*™ Neutralizing Ab ELISA.
- InBios International Inc., authorized distributors and authorized laboratories using the SCoV-2 *Detect*™ Neutralizing Ab ELISA must 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.

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

PERFORMANCE CHARACTERISTICS

Clinical Evaluation

The Clinical Evaluation tested 135 retrospectively collected serum samples, from subjects who had been confirmed positive for SARS-CoV-2 by an EUA-authorized RT-PCR assay. Samples were confirmed positive or negative for neutralizing antibodies by Plaque Reduction Neutralization Test at 90% reduction level ($PRNT_{90}$). The tables below show the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) between the SCoV-2 *Detect*™ Neutralizing Ab ELISA and the PRNT comparator assay. 95% confidence intervals (95% CI) were calculated by the Wilson score method.

		Plaque Reduction Neutralization Test ($PRNT_{90}$)	
		Positive	Negative
SCoV-2 *Detect*™ Neutralizing Ab ELISA	Positive	51	3
	Negative	2	79
	Positive Percent Agreement [95% CI]	96.2% [87.3%- 99.0%]	
	Negative Percent Agreement [95% CI]		96.3% [89.8%- 98.8%]

Cross-Reactivity (Analytical Specificity)

Cross-reactivity of the SCoV-2 *Detect*™ Neutralizing Ab ELISA Kit was evaluated by testing SARS-CoV-2 seronegative specimens from patients with antibodies to other viral infections and autoantibodies which could potentially cause false positive results. SCoV-2 *Detect*™ Neutralizing Ab ELISA demonstrates no cross-reactivity against antibodies for influenza A, influenza B, hepatitis B, hepatitis C, human immunodeficiency, respiratory syncytial viruses, anti-nuclear antibodies, dengue, zika, chikungunya, or antibodies against other human coronaviruses. Cross-reactivity was observed with high RF (>2000 IU/mL) samples, but did not correlate to RF levels.

Category	Number of samples tested	Number reactive
Anti-Influenza A/B	5	0
Anti-Hepatitis B	5	0
Anti-Hepatitis C	5	0
Anti-HIV	20	0
Anti-Nuclear Antibody	5	0
Anti-Dengue	8	0
Anti-Zika	8	0
Anti-Chikungunya	8	0
Anti-HKU1, OC43, 229E, NL63	5	0
Anti-Respiratory Syncytial Virus	4	0
Rheumatoid Factor	6	6

REFERENCES

Li, X., Geng, M., Peng, Y., Meng, L., & Lu, S. (2020). Molecular immune pathogenesis and diagnosis of COVID-19. *Journal of Pharmaceutical Analysis*. https://doi.org/10.1016/J.JPHA.2020.03.001

Okba, N. M. A., Muller, M. A., Li, W., Wang, C., GeurtsvanKessel, C. H., Corman, V. M., Lamers, M. M., Sikkema, R. S., de Bruin, E., Chandler, F. D., Yazdanpanah, Y., Le Hingrat, Q., Descamps, D., Houhou-Fidouh, N., Reusken, C. B. E. M., Bosch, B.-J., Drosten, C., Koopmans, M. P. G., & Haagmans, B. L. (2020). SARS-CoV-2 specific antibody responses in COVID-19 patients. *MedRxiv*, 2020.03.18.20038059. https://doi.org/10.1101/2020.03.18.20038059

Okba, N. M. A., Muller, M. A., Li, W., Wang, C., GeurtsvanKessel, C. H., Corman, V. M., et al. (2020). Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients. *Emerging Infectious Diseases,* 26(7).

Udugama, B., Kadhiresan, P., Kozlowski, H. N., Malekjahani, A., Osborne, M., Li, V. Y. C., Chen, H., Mubareka, S., Gubbay, J., & Chan, W. C. W. (2020). Diagnosing COVID-19: The Disease and Tools for Detection. *ACS Nano*. https://doi.org/10.1021/acsnano.0c02624

Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M.A., et al. (2020). Virological assessment of hospitalized patients with COVID-2019. *Nature*. https://doi.org/10.1038/s41586-020-2196-x

Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., Hu, Y., Tao, Z.-W., Tian, J.-H., Pei, Y.-Y., Yuan, M.-L., Zhang, Y.-L., Dai, F.-H., Liu, Y., Wang, Q.-M., Zheng, J.-J., Xu, L., Holmes, E. C., & Zhang, Y.-Z. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, *579*(7798), 265–269. https://doi.org/10.1038/s41586-020-2008-3

Yong, S.E.F., Anderson, D.E., Wei, W.E., Pang, J., Chia, W.N., Tan, C.W., et al. (2020). Connecting clusters of COVID-19: an epidemiological and serological investigation. *The Lancet Infectious Diseases*. https://doi.org/10.1016/S1473-3099(20)30273-5

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., … Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, *579*(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7

InBios International, Inc.
307 Westlake Ave N, Suite 300
Seattle, WA 98109 USA
1-866-INBIOS1 (Toll-Free, USA)
1-206-344-5821 (International)
www.inbios.com

Part Number: 900278-00
Effective Date: 10/22/2021

REF Catalog No. COVN-E