



Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit Instruction For Use

For Emergency Use Authorization (EUA) Only
 For *In vitro* Diagnostic (IVD) Use Only
 For Prescription Use Only

CONFIGURATION

The Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit is provided in the following kit configuration (100 tests) and is used on the fully automated DZ-Lite 3000 Plus Chemiluminescence Analyzer:

Component	Catalog # DZ901A
Magnetic beads	2.75 mL
Capture Reagent	12.5 mL
Detection Reagent	12.5 mL
Calibrator Low	0.5 mL
Calibrator High	0.5 mL
Positive Control	1.0 mL
Negative Control	1.0 mL

INTENDED USE

The Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit is a fully automated chemiluminescent immunoassay intended for the qualitative direct detection of total neutralizing antibodies to SARS-CoV-2 in human serum, dipotassium EDTA plasma and Lithium-Heparin plasma. The Diazyme SARS-CoV-2 Neutralizing Antibody CLIA 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 Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit should not be used to diagnose or exclude 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 or moderate 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 are 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 Diazyme SARS-CoV-2 Neutralizing Antibody CLIA 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 Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit is only for use under the Food and Drug Administration’s Emergency Use Authorization (EUA).

BACKGROUND

SARS-CoV-2 is a coronavirus that causes an acute respiratory syndrome in humans (COVID-19)¹⁻⁵. SARS-CoV-2 has been responsible for a global pandemic since March of 2020¹⁻³.

The immune system of patients infected with SARS-CoV-2 may produce antibodies against the virus. SARS-CoV-2 Neutralizing antibodies are a subset of SARS-CoV-2 antibodies that bind to the virus in a manner that inhibits viral endocytosis *in vitro*. A neutralizing antibody might block interactions with a cellular receptor or bind to a viral capsid in a manner that inhibits the uncoating of the viral genome⁴⁻⁵.

The Diazyme SARS-CoV-2 Neutralizing Antibody CLIA assay uses the SARS-CoV-2 Spike Receptor Binding Domain (RBD) and the human ACE2 receptor to assess the presence of neutralizing antibodies in a patient sample. The assay has high throughput and runs on a fully automated chemiluminescent analyzer. The assay is a faster and safer alternative to the low throughput cell-based assays that assess neutralizing antibodies using live viruses⁴⁻⁵.

ASSAY PRINCIPLE

The Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit is a competitive chemiluminescence immunoassay.

Sample, magnetic microbeads, capture reagent (containing SARS-CoV-2 Spike RBD) and a detection reagent (containing the human ACE2 receptor conjugated to ABEI) are mixed thoroughly and incubated, forming specific immune-complexes. After precipitation in a magnetic field and supernatant decanting, the magnetic particles are subjected to wash cycles. Subsequently, starter solutions are added to initiate a chemiluminescent reaction. In the presence of neutralizing antibodies in the sample, these antibodies bind to the viral antigen protein coated on the magnetic particles and block the interaction between the viral protein and the human ACE2 receptor, resulting in a decrease in the chemiluminescent signal (measured in RLU). The amount of the neutralizing antibody in a sample is inversely proportional to the RLU signal.

Positive and negative controls are provided with the kit to ensure the integrity of the assay. The assay is fully automated with the first results appearing after 34 minutes.

KIT CONTENTS

CARTRIDGE:

- Magnetic beads: Magnetic microbeads in PBS buffer supplemented with BSA and sodium azide (<0.1%)
- Capture reagent containing the SARS-CoV-2 Spike RBD in PBS buffer supplemented with BSA and sodium azide (<0.1%)
- Detection reagent containing a human ACE2 receptor in PBS buffer supplemented with BSA and sodium azide (<0.1%)
- Calibrator Low: recombinant SARS-CoV-2 neutralizing antibodies in pooled negative serum with sodium azide (<0.1%)
- Calibrator High: recombinant SARS-CoV-2 neutralizing antibodies in pooled negative serum with sodium azide (<0.1%)

CONTROLS:

- Negative Control: recombinant SARS-CoV-2 neutralizing antibodies in pooled negative serum with sodium azide (<0.1%)
- Positive Control: recombinant SARS-CoV-2 neutralizing antibodies in pooled negative serum with sodium azide (<0.1%)

OTHER:

- Cartridge with RFID card
- Sealing film
- Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

Analyzer	DZ-lite 3000 Plus CLIA Analyzer
Reaction Modules	REF: 630003
Starter 1 + 2	REF: 130299004M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M

STORAGE AND STABILITY

- Store at 2-8°C. Do not freeze.
- Keep away from light.
- The established stability of the kit is as follows:

Stability of the reagent	
Unopened at 2-8°C	Until the stated expiration date
Opened at 2-8°C	6 weeks
Onboard	4 weeks

To ensure the best kit performance, it is recommended to place opened kits in a refrigerator after the end of the work shift.

WARNINGS

- For Prescription and *In Vitro Diagnostic Use Only*.
- For Use under an Emergency Use Authorization Only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by the FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for detecting the presence of total 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.
- Human source material used to prepare the controls included in this kit should be handled as potentially infectious material. Use universal precautions when handling.
- Do not pipette by mouth
- Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards

SPECIMEN COLLECTION AND HANDLING

- Human serum as well as dipotassium EDTA or Lithium-heparin plasma can be used with this assay.
- Please pay attention to the risk of infection during sample

collection and preparation. Please refer to the CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19): <https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html> as well as your local, state and federal government’s mandated requirements.

- Ensure that complete clot formation in specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
- Do not use grossly hemolyzed specimens as well as specimens containing particulate matter or exhibiting obvious microbial contamination. Inspect all specimens for bubbles and remove bubbles before analysis.
- All samples (patient specimens and controls) should be tested within 3 hours of placing on board the analyzer. Refer to the instrument manual for more detailed discussion on onboard sample storage constraints.
- Specimens removed from the separator gel, cells or clot may be stored 3 days at 2-8°C.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. Specimens with particulate matter may give inconsistent results and must be transferred to a centrifuge tube and centrifuged at ≥ 10,000 RCF (Relative Centrifugal Force) for 10 minutes. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

PRECAUTIONS

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to blood borne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and container must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets, which are available upon request.

Handling Precautions

- Do not use reagent kits beyond their expiration date.
- Do not interchange kit components from different lots.
- Prior to loading the kit on the system for the first time, the kit requires mixing to re-suspend the magnetic microbeads that have settled during shipment.
- Wear clean gloves when operating with a reagent kit and sample.
- To avoid evaporation of the liquid in the opened reagent kits when stored in a refrigerator, it is recommended that the opened reagent kits be sealed with the provided sealing film.
- For detailed handling instructions of the analyzer, please refer to the Operating Manual.

ASSAY PROCEDURE

Step 1: Specimens Loading

- Pipette aliquots of the specimens to be tested (in singlicate) into clinical chemistry sample cups, barcoded test tubes or any standard

specimen testing tubes. Human serum, dipotassium EDTA plasma and Lithium-Heparin plasma can be used with the Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit.

- Load the samples to be tested on to the analyzer using the analyzer's sample racks. The analyzer will automatically detect the position of the sample rack(s).

Step 2: Loading of the Assay Cartridge

- Remove the assay cartridge from its box.
- Remove the sealing films attached to the five different compartments of the cartridge (magnetic beads, low calibrator, high calibrator, capture reagent and detection reagent).
- Scan the RFID card attached to the cartridge against the RFID reader located at the front of the analyzer. One short beep sound will indicate that the RFID card has been successfully read and that all assay parameters have been successfully transferred to the analyzer. Two short consecutive beeps mean that the RFID card was not read successfully and that it needs to be rescanned.
- Place the assay cartridge in any available slot of the DZ-Lite 3000 Plus reagent compartment (the system will automatically detect the location of the cartridge).
- Reagent information (assay name, lot number and calibration assigned values) will appear on the main page of the DZ-Lite 3000 Plus software interface.
- The analyzer will automatically start mixing/shaking the magnetic microbeads to ensure that they are a fully resuspended and homogenous before assaying. Resuspension should be allowed to proceed for at least 30 minutes prior to testing.

Step 3: Calibration

The Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit has been standardized against an internal reference material. A master calibration curve (RLU versus assigned neutralizing antibodies concentrations, in arbitrary units, AU/mL) is pre-encoded in the RFID card provided with the kit cartridge and is loaded onto the analyzer during step 2. This stored master curve is normalized to the actual signal of the analyzer of the day of testing by measuring the signal of two calibration verifiers (calibrator low and calibrator high) provided with the assay cartridge.

To perform calibration:

- Click the <Calibration> or <Batch Calibration> button to execute calibration. For specific information on ordering calibrations, refer to the Calibration Section of the Operating Manual.
- Daily recalibration is recommended AND if any of the following conditions occurs:
 - Each time a new reagent kit is used
 - After each exchange of lots (Reagent or Starter 1+2)
 - After instrument service
 - If controls fall outside the expected range

Step 4: Sample Testing

Navigate to the Sample tab of the user interface software and:

- Select the sample(s) to be tested.
- Press the button corresponding to the Diazyme SARS-CoV-2 Neutralizing Antibody application.
- Press the <Start> button.

For specific and detailed information on ordering patient specimens, refer to the Sample Ordering Section of the Operating Manual.

Quality Control

Two controls are provided with the Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit:

- **Negative Control:** This control contains recombinant SARS-CoV-2 neutralizing antibodies below the cut-off of the assay (< 1 AU/mL) in pooled human serum.
- **Positive Control:** This control contains recombinant SARS-CoV-2 neutralizing antibodies above the cut-off of the assay (≥ 1 AU/mL) in pooled human serum.

Diazyme recommends testing controls daily and each time there is an instrument service or a change in the Starter 1+2 solutions or the reagent cartridge. Furthermore, Diazyme recommends following government regulations or accreditation requirements for quality control frequency. Quality control samples should be treated and processed with the same level of care as patient samples. A satisfactory level of performance is achieved when obtained quality control results are within the pre-established ranges. If the quality control results are outside the pre-established ranges, quality controls should be repeated. If the quality control results still are outside the pre-established ranges, do not report results, and take the following actions:

- Verify that the control materials and the reagent cartridge are not expired.
- Verify that required instrument maintenance was performed.
- Verify that the assay was performed according to the instruction for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact Diazyme technical support for assistance.

INTERPRETATION OF RESULTS

All test controls should be examined prior to the interpretation of patient results. If the controls are outside the pre-established ranges, the run is considered invalid (expected range are indicated on the certificate of analysis of the test controls). Sample results from an invalid run should not be reported and samples should be retested.

The clinical applicability of the detection of SARS-CoV-2 neutralizing antibodies to the SARS-CoV-2 Spike antigen is currently unknown, and results cannot be interpreted as an indication of degree of immunity or protection from infection.

Results obtained with the Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit are interpreted and reported as indicated below:

Numerical Result (AU/mL)*	Result	Test Result Interpretation
x < 1.0	Negative	Neutralizing antibodies for SARS-CoV-2 are not detected**
x ≥ 1.0	Positive	Neutralizing antibodies for SARS-CoV-2 are detected**

* Numerical results observed with the Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit are not reported outside of the laboratory since this test is for qualitative use.

** The Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit results have shown 94.3% positive percent agreement (PPA) (95% CI 81.4-98.4%) and 98.7% negative percent agreement (NPA) (95% CI 93.2-99.8%) with 50% viral neutralization by PRNT in clinical study.

LIMITATIONS

- This test is designed for the qualitative detection of SARS-CoV-2 neutralizing antibodies.
- To be used only under the conditions of the FDA Emergency Use Authorization.
- Use of the Diazyme SARS-CoV-2 Neutralization Antibody CLIA Kit 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.
- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.
- The sensitivity of this assay early after infection is unknown.
- Negative results do not rule out SARS-COV-2 infection, particularly those who have been in contact with the virus. Direct testing with a molecular diagnostic should be performed to evaluate for acute SARS-CoV-2 infection in symptomatic individuals.
- A negative result can occur if the titer of neutralizing antibodies against the SARS-CoV-2 virus present in the specimen is below the sensitivity of the kit.

- False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
- Not for screening of donated blood.
- Results from this test should not be used to diagnose or to exclude acute SARS-CoV-2 infection or to inform infection status.
- It is unknown at this time if the presence of neutralizing antibodies to SARS-CoV-2 confers immunity to infection.
- The performance of this test 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 test 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 June 2020 to December 2020. Clinical performance has not been established in 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 LABORATORIES

The Diazyme DZ-Lite SARS-CoV-2 Neutralizing Antibodies CLIA 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/in-vitro-diagnostics-euas> Authorized laboratories using the Diazyme DZ-Lite SARS-CoV-2 Neutralizing Antibodies CLIA Kit must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

1. Authorized laboratories* using Diazyme DZ-Lite SARS-CoV-2 Neutralizing Antibodies CLIA Kit 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.
2. Authorized laboratories using Diazyme DZ-Lite SARS-CoV-2 Neutralizing Antibodies CLIA Kit 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.
3. Authorized laboratories that receive Diazyme DZ-Lite SARS-CoV-2 Neutralizing Antibodies CLIA Kit must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
4. Authorized laboratories using Diazyme DZ-Lite SARS-CoV-2 Neutralizing Antibodies CLIA Kit must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Diazyme Laboratories, Inc. (email: support@diazyme.com; 858-455-4768 Option 2) 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 Diazyme DZ-Lite SARS-CoV-2 Neutralizing Antibodies CLIA Kit 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. Diazyme Laboratories, Inc., and authorized laboratories using Diazyme DZ-Lite SARS-CoV-2 Neutralizing Antibodies CLIA Kit 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.
- *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 moderate or high complexity tests" as "authorized laboratories."

PERFORMANCE CHARACTERISTICS

Clinical Evaluation

Clinical evaluation of the Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit was performed with 114 retrospectively collected serum samples, obtained 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 a validated Plaque Reduction Neutralization Test (PRNT) at 50% reduction level (PRNT₅₀). The table below shows the Positive Percent Agreement (PPA) and negative percent agreement (NPA) between the Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit and the PRNT comparator assay. 95% confidence intervals (95% CI) were calculated according to the Wilson score method.

		PRNT Comparator Method	
		Positive	Negative
Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit	Positive	33	1
	Negative	2	78

PPA	94.3% (33/35)	95% CI = 81.4%-98.4%
NPA	98.7% (78/79)	95% CI = 93.2%-99.8%

Potential Cross-Reactivity (Analytical Specificity)

Potential Cross-reactivity of the Diazyme SARS-CoV-2 Neutralizing Antibody CLIA Kit was evaluated by testing SARS-CoV-2 negative specimens from patients with antibodies to other viral and bacterial infections. As shown in the table below the Diazyme assay did not cross react with any of the antibodies to the following pathogens: Influenza A Virus, Influenza B Virus, Hepatitis C Virus (HCV), Hepatitis B Virus (HBV), Haemophilus influenzae, Human Coronavirus 229E, Human Coronavirus NL63, Human Coronavirus OC43, Human Coronavirus HKU1, ANA, Respiratory Syncytial Virus, Human Immunodeficiency Virus (HIV) or Parainfluenza Virus.

Potential Cross-Reactants	Number of Samples Tested	Number of samples with Positive results
Influenza A Virus	10	0
Influenza B Virus	5	0
Hepatitis C Virus (HCV)	10	0
Hepatitis B Virus (HBV)	15	0
Haemophilus influenzae	10	0
Human Coronavirus 229E	5	0
Human Coronavirus NL63	5	0
Human Coronavirus OC43	5	0
Human Coronavirus HKU1	5	0
ANA	5	0
Respiratory Syncytial Virus (RSV)	5	0
Human Immunodeficiency Virus (HIV)	10	0
Parainfluenza Virus	5	0

REFERENCES

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