Diazyme Laboratories, Inc.
12889 Gregg Court
Poway, CA 92064, USA
Tel: 858-455-4768 / Fax: 858-455-3701
Email: **support@diazyme.com**
Website: www.diazyme.com

DIAZYME DZ-LITE SARS-CoV-2 IgG CLIA KIT

CONFIGURATION

The Diazyme DZ-Lite SARS-CoV-2 IgG CLIA Kit is provided in the following kit configuration (100 tests) and used in conjunction with the DZ-lite 3000 Plus fully automated chemiluminescence immunoassay instrument:

Component	Catalog # 130219015M
Magnetic Microbeads	2.5 mL
ABEI Label	23.5 mL
Diluent	23.5 mL
Buffer	23.5 mL
Negative Control	1.0 mL
Positive Control	1.0 mL
Calibrator Low	1.0 mL
Calibrator High	1.0 mL

INTENDED USE

The Diazyme DZ-Lite SARS-CoV-2 IgG CLIA Kit is for the detection of IgG antibodies against SARS-CoV-2 from human clinical specimen (serum or plasma).

• For Rx use only.

• This test has not been reviewed by the FDA. For use in clinical laboratories by health care professionals following FDA guidance "Policy for Diagnostic Tests for Coronavirus Disease-2019 (COVID-19) during the Public Health Emergency".

• Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic assay should be considered to rule out infection in these individuals.

• Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.

• 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 the screening of donated blood.

CLINICAL SIGNIFICANCE

The 2019-nCoV virus was first named by the World Health Organization on January 7, 2020. On February 11, 2020, the virus was renamed Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV). On the same day, the World Health Organization (WHO) announced that SARS-CoV-2-associated respiratory disease will be officially named COVID-19.

The novel coronavirus (SARS-CoV-2) that is causing an epidemic of acute respiratory syndrome in humans belongs to the family coronaviridae and the genus Betacoronavirus[1]. The virus has an envelope and its particles are round or oval, often polymorphic, with a diameter between 60 and 140 nm. The genetic characteristics of the virus are significantly different from those of SARSr-CoV and MERSr-CoV. Current research shows that SARS-CoV-2 has more than 85% homology with the bat SARS-like coronavirus (bat-SL-CoVZC45)[2].

SARS-CoV-2 is mainly transmitted through respiratory droplets and can also be transmitted through contact. The sources of infection seen mainly consist of patients with pneumonia infected by the novel coronavirus[2].

Research has shown that IgM and IgG antiviral antibodies can be detected in the serum samples of infected patients[3]. After infection with SARS-CoV-2, the virus antigen stimulates the immune system to produce antibodies that can be detected in the blood. Among these antibodies, SARS-CoV-2 IgM antibodies appears early and are mostly positive after 3-5 days of onset[2]. The SARS-CoV-2 IgM titers then decrease while the SARS-CoV-2 IgG antibody potency starts to rise rapidly. During the recovery phase, the titer of the SARS-CoV-2 IgG antibody may increase four times or more compared to the acute phase[2].

ASSAY PRINCIPLE

The Diazyme DZ-Lite SARS-CoV-2 IgG CLIA Kit is an indirect chemiluminescence immunoassay. The prediluted sample, buffer and magnetic microbeads coated with a SARS-CoV-2 recombinant antigen are mixed thoroughly and incubated, forming immune-complexes. After precipitation in a magnetic field and decanting of the supernatant, wash cycles are performed. Subsequently, ABEI-labeled anti-human IgG antibody is added and incubated to form additional complexes. After precipitation in a magnetic field and decanting of the supernatant, wash cycles are performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of SARS-CoV-2 IgG presented in the sample.

REAGENTS

- Magnetic Microbeads: Magnetic microbeads coated with SARS-CoV-2 recombinant antigen, PBS buffer and BSA, NaN_3(<0.1%).
- ABEI Label: Anti-human IgG antibody labeled with ABEI, Tris-HCl buffer, Mouse IgG, Goat IgG, and BSA, NaN_3 (<0.1%).
- Diluent: PBS buffer and BSA, NaN_3(<0.1%).
- Buffer: PBS buffer, Goat anti-Human IgG, Goat anti-Human IgA Mouse IgG, Goat IgG and BSA, NaN3 (<0.1%).
- Negative Control: PBS buffer, containing BSA, NaN3 (<0.1%).
- Positive Control: SARS-CoV-2 IgG, PBS buffer, containing BSA and NaN_3 (<0.1%).
- Calibrator Low: SARS-CoV-2 IgG, PBS buffer and BSA, NaN_3(<0.1%).
- Calibrator High: SARS-CoV-2 IgG, PBS buffer and BSA, NaN_3(<0.1%).
- All components of the kit are provided ready-to-use.

MATERIALS REQUIRED BUT NOT PROVIDED

DZ- Lite chemiluminescence 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 upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.
- The stability study is still on-going, the following data is obtained by referring to similar products:

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 the refrigerator after the end of the intraday test work.

SPECIMEN COLLECTION AND HANDLING

- Human serum or plasma may be used with the SARS-CoV-2 IgG CLIA Kit. For serum samples, standard sampling tubes, tubes containing separating gel or procoagulant inert separation tubes could be applied for the assay. For plasma samples, the anticoagulants including K_2-EDTA, K_3-EDTA, Na_2 -EDTA, were tested and found acceptable.
- Please pay attention to the risk of infection during sample collection and preparation. According to the "Diagnosis and treatment program of novel coronavirus pneumonia" issued in China, heat inactivation of the samples should be performed at 56°C for 30 minutes before testing[2]. 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.
- If the specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate substances.
- 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 for optimal results.
- All samples (patient specimens and controls) should be tested within 3 hours of placing on board the DZ-Lite System. 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. If longer storage is required, freeze the specimens at -20°C or colder[4].
- Avoid repeated freezing and thawing. Frozen specimens must be mixed thoroughly after thawing by low speed vortexing or by gently inverting.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens 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.
- Before shipping specimens, it is recommended that specimens be removed from the separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable local, state, federal and international regulations covering the transport of clinical specimens and infectious substances. It is recommended specimens should be shipped frozen.

- The sample volume required for a single determination is 10 µL.

PRECAUTIONS

- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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 bloodborne 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 on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the Reagent Kit on the system for the first time, the Reagent Kit requires mixing to re-suspend magnetic microbeads that have settled during shipment. For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are "single use".
- For detailed discussion of handling precautions during system operation, refer to the instrument manual.

WARNINGS

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[5].

ASSAY PROCEDURE

Preparation of the Reagent

- Take the reagent kit out of the box and check the sealing film and other parts of the reagent kit for any signs of leakage. In case of leakage, please contact your local distributor immediately. Tear off the kit sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight, insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended and homogenous prior to use. Resuspension should be allowed for at least 30 minutes prior to testing samples.

Assay Calibration

- Click **<Calibration>** or **<Batch Calibration>** button to execute calibration operation. For specific information on ordering calibrations, refer to the Calibration Section of the Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Traceability

This method has been standardized against an internal reference substance. Test of assay-specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent Radio Frequency Identification (RFID) CHIP. Recalibration is recommended if any of the following conditions occurs:

- After each exchange of lots (Reagent or Starter 1+2).
- Every week and/or each time a new reagent kit is used.
- After instrument service.
- If controls lie outside the expected range

Quality Control

Follow government regulations or accreditation requirements for quality control frequency. Internal quality control is only applicable to this system. User needs to judge the obtained results with their own standards and knowledge. For details about entering quality control values, refer to the operating instructions of the fully automated chemiluminescence immunoassay analyzer. To monitor system performance, quality control materials (negative control and positive control) are required. Treat all quality control samples with the same level of care as patient samples. A satisfactory level of performance is achieved when obtained analyte values are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Range or within the laboratory's established Range, measurement of the quality control should be repeated. If the quality control results still do not fall within the range, do not report results and take the following actions:

- Verify that the materials are not expired.
- Verify that required 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 your local technical support provider or distributor for assistance.

In order to avoid manual error in the entry of QC information, the provided barcode labels of quality control can be used attached to the test tubes.

If users do not use the provided barcode labels for positive and negative controls contained within the packaging, then quality controls should be entered manually. For specific information on ordering quality controls, refer to the Quality Control Section of the Operating Instructions.

Sample Testing

Order the samples in the Sample Area of the software and click the **<Start>** button to execute testing. For specific information on ordering patient specimens, refer to the Sample Ordering Section of the Operating Instructions.

To ensure proper test performance, strictly adhere to the operating instructions of the fully automated chemiluminescence immunoassay analyzer.

Dilution

High concentration samples can be diluted automatically by the analyzer or manually.

After manual dilution, multiply the result by the dilution factor. After dilution by the analyzer, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

The automatic sample dilution is available after dilution settings are checked in the fully automated chemiluminescence immunoassay analyzer user software. Please refer to the operating instructions of the fully automated chemiluminescence immunoassay analyzer.

RESULTS

Calculation of Results

The analyzer automatically calculates the concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in AU/mL. For further information please refer to the operating instructions of fully automated chemiluminescence immunoassay analyzer.

Reporting of Results

Result reports must include the following statement:

- This test has not been reviewed by the FDA.
- Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
- Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
- 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 the screening of donated blood

REFERENCE RANGE

A reference range study in China using the Diazyme DZ-Lite SARS-CoV-2 IgG assay yielded the following results:

- Non-reactive: A result less than 1.00 AU/mL (<1.00 AU/mL) is considered to be non-reactive.
- Reactive: A result greater than or equal to 1.00 AU/mL (≥1.00 AU/mL) is considered to be reactive.
- For samples with concentration near the cut-off or positive, follow-up tests should be performed. If the antibody level does not change significantly, patient's viral nucleic acid results and imaging features such as CT (Computed Tomography) should be combined for confirmed diagnose.
- Results may differ between laboratories due to variations in population. It is recommended that each laboratory establish its own expected ranges.

LIMITATIONS

- This test is suitable only for investigating single samples, not for pooled samples.
- Bacterial contamination or repeated freeze-thaw cycles may affect the test results.
- Assay results should be utilized in conjunction with other clinical and laboratory methods to assist the clinician in making individual patient diagnostic decisions.
- Assay results should not be used as the sole basis for the diagnosis and exclusion of novel coronavirus pneumonia, but only as a supplement to existing viral nucleic acid detection reagents and imaging features.
- It is recommended to be used in conjunction with SARS-CoV-2 IgM testing to improve clinical sensitivity.
- If the SARS-CoV-2 IgG results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- HAMA antibodies in test samples may cause interference in immunoassays.

PERFORMANCE CHARACTERISTICS

Precision

Precision for SARS-CoV-2 IgG CLIA Kit was determined as described in the CLSI EP5-A3. 2 controls and 3 samples containing different concentrations of analyte were assayed in duplicate at three sites over five days, with 3 runs per day, one lot of reagent for each run and 2 replicates per run. The results are summarized in the following table:

Sample	Mean Value (AU/mL)	N	Repeatability		Between-Lot		Between-Day		Between-Site		Reproducibility	
			SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV
NQC	0.293	90	0.024	NA	0.005	NA	0.008	NA	0.023	NA	0.035	NA
PQC	3.915	90	0.199	5.08	0.069	1.76	0.032	0.82	0.265	6.77	0.340	8.68
S1	0.491	90	0.043	NA	0.015	NA	0.004	NA	0.013	NA	0.047	NA
S2	3.486	90	0.212	6.08	0.060	1.72	0.050	1.43	0.071	2.04	0.237	6.80
S3	9.807	90	0.159	1.62	0.122	1.24	0.082	0.84	0.639	6.52	0.675	6.88

Interference
Two samples (one negative sample, one positive sample) were spiked with potential endogenous interference and exogenous interference substances. The results are listed in the following table:

Substance	No interference up to
Bilirubin	40 mg/dL
Triglycerides	1000 mg/dL
Hemoglobin	2000 mg/dL
Rheumatoid Factor	1500 IU/mL
Anti-Mitochondrial	1:64(titer)
HAMA	30 ng/mL
Total IgG	1600 mg/dL
Total IgM	280 mg/dL
Interferon α	1500 U/mL
Ribavirin	90 mg/dL
Oseltamivir	1.0 mg/dL
Levofloxacin	1.776 mg/dL
Azithromycin	1.201 mg/dL
Ceftriaxone sodium	81.03 mg/dL
Meropenem	80.15 mg/dL
Tobramycin	2.4 mg/dL
Diphenhydramine Hydrochloride	4.5 mg/dL
Oxymetazoline	2.5 mg/dL
Sodium chloride	45 mg/dL
Beclomethasone	2.5 mg/dL
Dexamethasone	18 mg/dL
Triamcinolone acetonide	5.5 mg/dL
Budesonide	3.2 mg/dL
Mometasone	2.5 mg/dL
Fluticasone propionate	2.5 mg/dL

Cross-Reactivity
The cross-reactivity study for the SARS-CoV-2 IgG CLIA Kit was designed to evaluate potential cross-reactants. The results are listed in the following table:

Condition	N of samples containing cross-materials	N of SARS-CoV-2 IgG assay positive results
Influenza A virus antibodies	17	0
Influenza B virus antibodies	19	0
Parainluenza virus antibodies	23	0
Respiratory syncytial virus antibodies	7	0
Adenovirus antibodies	9	0
EBV NA IgG	10	0
EBV VCA IgG	4	0
EBV VCA IgM	6	0
Mealles virus	2	0
CMV IgG	6	0
CMV IgM	2	0
Varicella zoster virus antibodies	2	0
M.Pneumonia IgG	3	0
M.Pneumonia IgM	4	0
Chlamydia pneumoniae IgG	3	0
Chlamydia pneumoniae IgM	3	0
Monilia albican	1	0
ANA	6	0
SARS-CoV-2 IgM	6	0
Total	133	0

Clinical Sensitivity
The clinical sensitivity was determined in China using confirmed novel coronavirus infected specimens. The clinical sensitivity for SARS-CoV-2 IgG assay was found to be 91.21%. When used in combination with the SARS-CoV-2 IgM assay the clinical sensitivity was found to be 95.60%.

Specimen Category	SARS-CoV-2 IgG CLIA			SARS-CoV-2 IgM CLIA + SARS-CoV-2 IgG CLIA		
	N	Positive	%Sensitivity	N	Positive	%Sensitivity
Clinical confirmed positive samples	91	83	91.21	91	87	95.6

Clinical Specificity
The clinical specificity was determined in China using non-novel coronavirus infected specimens (including normal samples and interference samples). The clinical specificity for SARS-CoV-2 IgG Kit was found to be 97.33%. When used in combination the SARS-CoV-2 IgM the clinical specificity was found to be 96.00%.

Specimen Category	SARS-CoV-2 IgG CLIA			SARS-CoV-2 IgM CLIA + SARS-CoV-2 IgG CLIA		
	N	Negative	%Specificity	N	Negative	%Specificity
negative specimens	750	730	97.33	750	720	96

REFERENCES

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Diazyme Laboratories, Inc.
12889 Gregg Court
Poway, CA 92064, USA
Tel: (858) 455-4754
Fax: (858) 455-4750
support@diazyme.com