BioCLIA® Autoimmune Reagent Kit

dsDNA

Chemiluminescent Microparticle Immunoassay

Magnetic bead chemiluminescence immunoassay (CLIA) for quantitative determination of anti-dsDNA antibody in human serum/plasma

Key to Symbols Used

REF	Catalog Number		Expiration Date
IVD	For *In Vitro* Diagnostic Use	LOT	Lot Number
	Store between +2°C and +8°C		Consult Instruction for Use
	Manufacturer	EC REP	Authorized Representative in European Union
ANTIGEN BIOT	Biotinylated Antigen	Σ	ContainsSufficient for < *n* > Tests
CONJ AP G	Conjugate		Chemical Risk Warning
M STREP	Microparticle		Biological Risk Warning

BioCLIA® Autoimmune Reagent Kit, dsDNA

Intended Use

BioCLIA dsDNA assay is intended for the *in vitro* quantitative measurement of IgG antibodies directed against double stranded deoxyribonucleic acid (dsDNA) in human serum/plasma as an aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and related autoimmune diseases. This kit is used on the instrument of BioCLIA® 1200 and BioCLIA® 6500.

Catalog Numbers

MY00097 (50 Tests/kit)
MY00148 (100 Tests/kit)

Summary and Explanation

Anti-nuclear antibody (ANA) is a class of auto-antibodies with different binding affinities specific to different nuclear antigens. Generally, ANAs include extractable nuclear antigen (ENA) antibodies and un-extractable nuclear antigen antibodies. 1 Determination of ANA has significant correlation to the diagnosis of Sharp syndrome (MCTD), SLE, sjogren's syndrome, progressive systemic sclerosis, polymyositis/dermatomyositis, overlap syndrome, and limited types of progressive systemic sclerosis (CREST syndrome). Anti-DNA antibody can be divided into two types: anti-double-stranded DNA (dsDNA) antibodies and anti-denatured single-stranded DNA (ssDNA) antibodies. Anti-dsDNA antibody can bind to the double helix of DNA skeleton while ssDNA antibody only interacts with purine or pyrimidine base polymers.2, 3 Anti-dsDNA antibodies are recognized as the major serologic marker of SLE. Their specificity and their sensitivity give them a high diagnostic value. Therefore they are a part of the clinical and biological criteria established in 1982 by the American Rheumatism Association (ARA) for the diagnosis of the SLE. The detection of anti-dsDNA antibodies is particularly useful in two different ways: as an aid to the diagnosis of SLE and as a tool to monitor the course of the disease. For the second purpose repeated serum sampling of individual patients can be very informative about the clinical course of the disease because a clear-cut relationship exists between anti-DNA and diseases activity : flares of SLE are generally preceded by a rise in anti-dsDNA levels, followed by a steep drop during the exacerbation (particularly in nephritis). Furthermore different treatments of patients have varying influences on anti-dsDNA levels and can be adapted by a regular follow-up of these antibodies.

Principles of the Procedure

BioCLIA dsDNA assay is a two-step immunoassay using microparticle enzyme-labeled chemiluminescent technology.

In the first step, the streptavidin coated magnetic microparticle, the biotinylated dsDNA and human serum/plasma sample are mixed in an assay tube, which allows patient specific anti-dsDNA to bind. Secondly, after incubation, unbound biotinylated dsDNA and sample matrix are removed by washing, and the Microparticle-dsDNA-anti-dsDNA antibodies immune complexes are kept with the help of a magnetic separator.Third, anti-human IgG conjugated alkaline phosphatase is added. Fourth, after incubation, excess enzyme conjugates are removed by washing and finally the bound enzyme is detected by addition of chemiluminescent substrate. The relative light unit (RLU) intensity is proportional to the amount of anti-dsDNA specific IgG. According to the anti-dsDNA specific IgG RLU-concentration standard curve, the RLU tested can be interpreted to anti-dsDNA specific IgG concentration in the sample expressed as IU/mL.

For quantitation of anti-dsDNA antibodies, the BioCLIA dsDNA assay utilizes a predefined lot-specific Master Curve that is uploaded into the instrument through the reagent Master Calibration Curve barcode. The Master Curve is created during manufacturing by using in-house standards that are traceable to the First International Standard Preparation for dsDNA (WHO code: Wo/80). Based on the Master Curve and results obtained by running two Calibrators, an instrument specific Working Curve is created, which is used to calculate International Units (IU)/mL from the RLU obtained for each sample.

Specimen Collection

The appropriate specimen types for BioCLIA dsDNA are human serum and plasma (Sodium citrate

anticoagulant; Heparin anticoagulant; EDTA anticoagulant).Cloudy samples should be purified by low-speed centrifugation.To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increasing clotting time.

Freshly collected specimensstored in refrigerator (2-8 °C) are valid for testing within 8 days. The stored specimen should reach to room temperature (18-25°C) before testing, and should not be stored in this temperature condition more than 2 days. All on board specimens should be testedwithin 10 hours. Three freeze (at -20°C) -thaw cycles for specimens do not affect the testing results.

Warnings and Precautions

1. This assay is only for use in the BioCLIA® 1200 and BioCLIA® 6500.

2. This product requires the handling of calibrators, controls and human specimens which contain human sourced materials.It is recommended that all human sourced materials are considered to be potentially infectious and handled in accordance with the OSHA Standardon Bloodborne Pathogens.[8] Biosafety Level 2[9] or other appropriatebiosafety practices [10, 11]should be used for materials that contain or possibly contain infectious agents.Avoidcontacting with skin and eyes. Wear suitable protective gloves and clothing.

3. Liquid waste and solid waste are temporarily stored at separate containers. Waste management should also be handled in accordance with standards mentioned in chapter Warnings and Precautions point No. 2.

4.Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing wastes.

5. Once opened, this reagent cartridge must be stored in the instrument's reagent carousel. Avoid spilling the reagents when the reagent cartridge is placed into the instrument.

6. Chemical contamination of the reagents can resulting from improper cleaning or rinsing of the instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the BioCLIA® 1200 and BioCLIA® 6500 operator.

7. Precautions: 

Proclin 300 added in the reagents (dsDNA Antigen, Conjugate) at concentration between 0.0015% - 0.6%.

StorageInstructions

The kit is stable until the expiration date, if it is stored and handled as directed. Routine store the kit in refrigerator (2-8°C). Vial openedreagents or onboard reagentscan be used up to 28 consecutivedays (2-8°C). The BioCLIA® 1200 and BioCLIA® 6500 software monitors the expiration of the reagent cartridge. Thesystem will not accept expired reagents. Three freeze (at -20°C) -thaw cycles before testing has no effect on the kit reagents.

Materials Supplied

Components are matched in sets. Labels supplied within the kit will be needed for the assay testing.

- **dsDNA Antigen** One bottle (2.5/5 mL) Biotinylated dsDNA antigen in 0.01M PBS(pH7.4) buffer with stabilizer. dsDNA | BIOT

Preservatives: 0.0015% < Proclin 300 < 0.6%.

- **Conjugate** One bottle (6.75/13.5 mL) AP labeled anti-human IgG antibodies in 0.05M MES(pH6.0) Buffer with stabilizer. CONJ | AP | G

Preservatives: 0.0015% <Proclin 300 < 0. 6%.

- **Microparticle** One bottle (2.5/5 mL) Streptavidin-microparticles in 0.01M PBS(pH7.4) buffer with stabilizer. M | STREP

Preservatives: 5-Bromo-5-Nitro-1,3-Dioxane (BND) <1%.

Kit Component Supplied Separately

Additional Materials Required But Not Provided:

- BioCLIA®1200 (Cat No. MA00139)
- BioCLIA®6500 (Cat No. MA00243)
- BioCLIA Autoimmune Calibrator Set, dsDNA (Cat No. MY00199, 2 x 1 mL; Cat No. MY00250, 4 x 1 mL)
- BioCLIA Autoimmune Control Set, dsDNA (Cat No. MY00301, 2 x 1 mL; Cat No. MY00352, 4 x 1 mL)
- BioCLIA Sample Diluent I (Cat No. MY00965)
- BioCLIA System Wash Buffer (Cat No. MY00404)

- BioCLIA System Substrate (Cat No. MY00405)
- BioCLIA Cuvettes (Cat No. MA00138, MA00244)
- BioCLIA Silicon Gasket (Small) (Cat No. MV00195)
- BioCLIA Silicon Gasket (Large) (Cat No. MV00196)
- BioCLIA Substrate Tube Maintenance cleanser (Cat No. MA00140)
- BioCLIA Sample Probe Maintenance cleanser (Cat No. MA00141)
- BioCLIA Micro Cup (Cat No. MA00142)
- Distilled Water

Assay Procedure

Note that, it is important to perform all routine maintenance procedures for optimal performance, such as routine cleaning, calibration and control procedures that are defined in the BioCLIA® 1200 and BioCLIA® 6500 User Manual.

See the BioCLIA® 1200 and BioCLIA® 6500 User Manual forpreparation, setup, dilutions, adjustment, assay and quality control procedures.

Users should have the periodic calibration procedure for every 28 consecutive days from last calibration. Besides, a calibration procedure should be carried out when a new batch of BioCLIA dsDNA kit is used.

The control procedure should be done before running the specimens each day. Users also can adjust the control procedure period according to their own lab frequency.

Expected Values

Each Laboratory should establish its own reference ranges.

When the customer see a problem (High CV or unusual values, rerun controls and analyze specimens again.

Result Analysis

With the help of the built in master calibration curve and specifiedtwo-point calibrator set for the instrument, the BioCLIA will automatically calculate the auto-antibodies concentration of each specimen and interpret the results into IU/mL. The concentration of anti-dsDNA antibody sample is reported as <1 IU/mL when it is lower than the minimum detection limit, while reported as > 800 IU/mL when it is higher than the range of measurement.

Sample Dilution

The specimens are dilutedwith BioCLIA Sample Diluent I before testing (dilution ratio 1:20) by the BioCLIA® 1200 and BioCLIA® 6500 automatically.

Cut-Off Value Determination

120 clinical samples, including 30 positive sera, 30 negative sera, 30 positive plasma and 30 negative plasma were collected and valued. These samples were venous blood from human aged between 0 - 80, sealed and stored at 2 - 8 ^{o}C.Results of 120 clinical samples tested by the BioCLIA dsDNA kit were analyzed using the receiver-operating characteristic curve (ROC) and the cut-off value was determined at 10 IU/mL.

Test Result Interpretation

Specimen with concentration < 10 IU/mL, interpreted as negative;

Specimen with concentration ≥ 10 IU/mL , interpreted as positive.

Test results only reflect the sample collecting status and should be interpreted/analyzed for diagnosis in conjunction with other laboratory and clinical findings.

Performance Characteristics

APPEARENCE

Kit components are complete with no leakage. No precipitation or floc in liquid reagents. Packing labels are clear and easy to be identified.

ACCURACY / SPIKED RECOVERY

The accuracy/spiked recovery was determined by analyzing samples spiked with known amounts of anti-dsDNA antibodies into certain matrix. Anti-dsDNA antibody positive samples (low 100 IU/mL, mid 200 IU/mL, mid 300 IU/mL, high 700 IU/mL) were spiked into twomatrixes (50 & 100 IU/mL) separately at the volume ratio of 1:9, making totally 8 spiked samples and each sample was tested in triplicate. The spiked recovery for the concentration of anti-dsDNA antibodies was calculatd.*

	Matrix 50 IU/mL			Matrix 100 IU/mL		
Spiked Conc.	**Obs**	**Exp.**	**Obs/Exp**	**Obs**	**Exp.**	**Obs/Exp**
Neat	48.21			92.22		
100 IU/mL	48.99	55	89.07%	90.59	100	90.59 %

200 IU/mL	63.84	65	98.22%	98.70	110	89.73 %
300 IU/mL	72.64	75	96.86%	113.9 5	120	94.96 %
700 IU/mL	119. 98	11 5	104.33 %	156.7 4	160	97.96 %

*Representative data; results in individual laboratories may vary from these data.

TRACEABILITY

Concentration of Anti-dsDNA antibody can be traced to WHO International Standard Preparation for dsDNA, NIBSC Code:wo/80.

PRECISION

A study based on guidance from CLIS document EP5-A2was performed for determining the precision of BioCLIA dsDNA kit. Human serum in the in-house reference panel (RP1, RP2, RP3, RP4) was tested with 10 replicates per sample for intra-assay precision evaluation, while with 4 replicates per sample for inter-assay precision. Each sample tested in individual runs, and 2 runsper day for 10 days, a total of 80 points. Data from this study are summarized in the following table.*

Intra-assay precision: CV <10%

Intra-Assay	**RP1**	**RP2**	**RP3**	**RP4**
Mean(IU/ml)	10.11	21.36	93.19	699.58
CV	2.2%	5.1%	3.5%	1.8%

Inter-assay precision: CV <15%

Inter-Assay	**RP1**	**RP2**	**RP3**	**PR4**
Mean(IU/ml)	9.77	19.97	97.40	706.45
CV	3.6%	7.5%	5.5%	2.6%

*Representative data; results in individual laboratories may vary from these data.

LIMIT OF BLANK / DETECTION (LOB/LOD)

LOB/LOD was determined consistent with CLSI EP17-A guideline.LOB/LOD of the BioCLIA dsDNA assay was lower than 1.0 IU/mL, which is below the analytical measuring range of the assay.

ASSAY REPORTABLE RANGE

The BioCLIA dsDNA kit has a reportable linear range of 1 - 800 IU/mL. The linear range was determined by diluting a high positive anti-dsDNA antibody serum sample with a negative sample to several concentrations which covers the entire assay linear range according to the scheme in CLSI EP6-A. The expected value was plotted against the observed value, and linear regression analysis was performed to get slope, intercept and coefficient of correlation (r) values. The results are summarized in the tables below:*

Slope	**Intercept**	**r**
1.00	-1.10	0.99

Assay linear range is 1-800 IU/mL. Results below the lower limit will be reported as < 1 IU/mL, while those are above the upper limit will be reported as > 800 IU/mL.

*Representative data; results in individual laboratories may vary from these data.

INTERFERENCE

Bilirubin, hemoglobin, triglycerides, rheumatoid factor (RF), and human anti-mouse antibody (HAMA) will not affect the BioCLIA dsDNA assay performance when at the level indicated below.

Bilirubin ≤40 mg/dL;

Hemoglobin ≤150 mg/dL;

Triglycerides≤1,000 mg/dL;

Rheumatoid factor (RF) ≤1,000 IU/mL;

Human anti-mouse antibody (HAMA) ≤2,000 ng/mL.

METHOD COMPARISON

Method comparison was implemented by comparing BioCLIA dsDNAassay to the predicated assay.

Clinical Sample		**BioCLIA dsDNA**		
		-	**+**	**Total**
Predicated Method	**-**	49	1	50
	+	6	44	50
	Total	55	45	100

Sensitivity	88.0%
Specificity	98.0%
Total agreement	93.0%

Limitations

•The effectiveness of this kit is only confirmed for human serum/plasma, the applicability of the other kinds of samples is not verified.

•Reliable and reproducible results will be obtained when the assay procedure is carried out in accordance with the instructions and with adherence to good laboratory practice.

•Clinical diagnosis should not be made on the findings of a single test result, but should be integrated with all clinical and laboratory findings.

Wayne, PA: Clinical andLaboratory Standards Institute, 2005.

References

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Technical Assistance

For technical assistance, contact your National Distributor.

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