BioCLIA Autoimmune Reagent Kit, ICA

(Chemiluminescent Microparticle Immunoassay)

Kit size	Cat. No.	GTIN Code
50 Tests/kit	MY00144	06924030402594
100 Tests/kit	MY00195	06924030403102

INTENDED USE

The BioCLIA Autoimmune Reagent Kit, ICA assay is intended for the in vitro quantitative measurement of antibodies directed to ICA in human serum as an aid in the diagnosis of Insulin-dependent diabetes mellitus (IDDM), Type 1 in conjunction with other laboratory and clinical findings. It is an *in vitro* diagnostic medical device intended for laboratory professional use.

SUMMARY AND EXPLANATION OF THE TEST

Insulin-dependent diabetes mellitus (IDDM), Type 1, is caused by the autoimmune destruction of the beta cells in pancreas. [1, 2] This selective autoimmune pathogenesis causes complete disruption of insulin secretion. The immunological evidence was demonstrated by the presence of specific islet cell autoantibodies in IDDM sera. [3] At least three autoantibodies have been identified against antigenic components of the islet cells in Type 1 diabetics. These autoantibodies are directed specifically to islet cell antigenic components, glutamic acid decarboxylase and insulin. [4]

Islet Cell Autoantibodies are present in 70% of patients with a recent onset of IDDM compared with 0.1-0.5% of the control non-diabetic population. [5] ICA are also detected in first degree relatives of IDDM patients. These individuals comprise the segment of human population who are at a high risk of developing IDDM. Several studies reported that the ICA-positive first degree relatives of IDDM patients subsequently suffered from diabetes. [6] Other studies also suggested that the presence of serum ICA and IAA is an indicator of the enhanced likelihood to develop IDDM. [7] Therefore, serological detection of ICA may be a powerful tool for early diagnosis of IDDM. The significance of these autoantibodies as markers of IDDM is also illustrated by their presence in nondiabetic individuals who ultimately develop IDDM. The screening of high-risk populations, for all of these three autoantibodies (ICA, IAA and ICA) will help to either prevent or to slow down the onset of the disease. A high-risk (asymptomatic) population, positive for two or more autoantibodies, is vulnerable for developing IDDM, usually in the next 5-7 years. [8]

PRINCIPLES OF THE PROCEDURE

BioCLIA Autoimmune Reagent Kit is a two-step immunoassay. In the first step, the streptavidin coated magnetic microparticle, the biotinylated antigen and human serum/plasma sample are mixed in an assay tube, which allows patient specific anti-antigen to bind. Secondly, after incubation, a washing step removes the unbound and unspecific bound molecules. Subsequently add enzyme labeled anti-human IgG antibodies and bind to the IgG-antigen complex. After incubation, a second washing step removes the unbound enzyme-IgG-antigen complex. Then addition of substrate results in the emission of light and the relative light unit (RLU) intensity is measured. The relative light unit (RLU) intensity is proportional to the amount of antigen specific IgG present in the sample.

The BioCLIA Autoimmune Reagent Kit utilizes a predefined lot specific Master Curve which is uploaded into the instrument via the barcode provided in the kit. Based on the results of running two calibrators, the instrument specific Working Calibration Curve is generated and is used to calculate the concentration from the RLU obtained for each patient.

WARNINGS AND PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Used on BioCLIA 6500 and BioCLIA 500 instruments only.
- Do not use reagents beyond the expiration dates.
- The kit contains human sourced materials. All recommended precautions for the handling of blood derivatives should be taken. Please refer to the existing laboratory safety regulations and good laboratory practice.
- Liquid waste and solid waste taken from BioCLIA 6500 and BioCLIA 500 should also be handled in accordance with the National or Local legislation.
- Once opened, the reagent cartridge must be stored in the instrument's reagent carousel. For the first placement of reagent into the instrument, please take care to avoid spilling the reagents.
- Spilled reagents should be cleaned up immediately. Comply with all National and local environmental regulations when disposing of wastes.

  Improper cleaning or rinsing of the instrument may lead to chemical contamination of the reagents. 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 as outlined in the BioCLIA 6500 and BioCLIA 500 User's Manual.

Precautions

- The assay contains ProClin 300 0.0015%~3% as preservative and may cause an allergic skin reaction by skin contact. Avoid contact with skin. Wear protective gloves, protective clothing and protective glasses.
- The assay contains TritonX-100 0.0015%~0.5% as surfactant and may cause an allergic skin reaction by skin contact. Avoid contact with skin. Wear protective gloves, protective clothing and protective glasses.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the

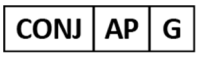
patient is established.

MATERIALS SUPPLIED

• **Buffer** 0.01M PBS (pH7.4).

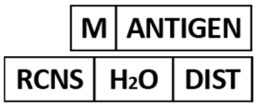
Preservatives: 0.0015%＜ProClin 300 < 3%.
Surfactant: 0.0015%＜Triton X-100 < 0.5%.

• **Conjugate** Alkaline phosphatase (AP) labeled anti-human IgG antibodies in 0.05 M MES (pH6.0) Buffer with stabilizer.



Preservatives: 0.0015%＜ProClin 300 < 3%.

• **Microparticle** Lyophilized antigen coated on microparticles in 0.02M Tris (pH 8).



Preservatives: 0.0015%＜ProClin 300 < 3%.

Components	50 Tests/Kit	100 Tests/Kit
Buffer	1 x 2.5 mL	1 x 5 mL
Conjugate	1 x 6.75 mL	1 x 13.5 mL
Microparticle	1 x sq 4 mL*	2 x sq 4 mL*

**sq 4mL: Each bottle reconstituted with 4mL distilled water before using.*

ADDITIONAL MATERIALS SUPPLIED SEPARATELY

Product	CATALOGUE No.
BioCLIA Autoimmune Calibrator Set, ICA	MY00246 (2 x 1 mL) MY00297 (4 x 1 mL)
BioCLIA Autoimmune Control Set, ICA	MY00348, (2 x 1 mL) MY00399 (4 x 1 mL)
BioCLIA Sample Diluent I	MY00965
BioCLIA System Wash Buffer	MY00404
BioCLIA System Substrate	MY00405
BioCLIA 6500	MA00243
BioCLIA 500	MA00502
BioCLIA Cuvettes	MA00244 (2000 pcs/bag) MA00549 (65 pcs/box)
BioCLIA Silicone gasket (Small)	MV00195
BioCLIA Silicone gasket (Large)	MV00196

MATERIALS REQUIRED

- Distilled or deionized Water

STORAGE AND STABILITY

- Store the kit at 2-8 °C.
- The shelf life of the unopened kit is 12 months.
- Opened reagents or onboard reagents may be used for 28 successive days. The software of the BioCLIA instruments monitors the onboard (in-use) expiration of the reagent cartridge. The system will not allow use of a reagent which has expired.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Serum from venous can be used.
- Collect blood specimens using standard procedures.
- Test serum should be clear and free from hemolysis.
- Cloudy samples should be clarified by centrifugation at 5000 rpm for 5 minutes before use. For samples with 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 increased clotting time.
- Specimens may be refrigerated at 2-8 °C for up to seven days or stored at -20°C up to six months.
- Specimens may be kept onboard on BioCLIA instruments under room temperature (18-25°C) for up to 2 hours.
- Avoid repeated freezing and thawing.

ASSAY PROCEDURE

Detailed information about operating the BioCLIA instruments can be taken from the Instrument User's Manual.

Note that, it is important to perform all routine maintenance procedures for optimal performance.

Lyophilized Microparticle Reconstitution

Reconstitute the lyophilized antigen coated microparticles with distilled water (4mL distilled water per bottle). Blend for more than 30 minutes in low speed, and transfer the solution to the supplied empty microparticle bottle. For the kit size of 100 Tests/Kit, user should transfer both bottles of reconstitute microparticle solution to supplied empty microparticle bottle before using.

Sample Dilution

The specimens are diluted with BioCLIA Sample Diluent I before testing (dilution ratio 1:19) by the BioCLIA instruments automatically.

Assay Calibration

The BioCLIA Autoimmune Reagent Kit utilizes a predefined lot specific Master Curve which is uploaded into the instrument via the barcode provided in the kit. Based on the results of running two calibrators, the instrument specific Working Calibration Curve is generated and is used to calculate the concentration from the RLU obtained for each patient.

For each new lot of reagent, please calibrate prior to the first time use, and every 28 days thereafter. The software will not allow the lot to be used if the above requirements are not meet.

Control

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.

Each Laboratory should establish its own reference ranges.

Programming and Running samples

1. Put the kit into any empty position of the reagent chamber of the BioCLIA instruments. Details of the kit can be uploaded into the instrument system through the scanning of reagent barcode, and can also be set manually.
2. The information of calibrator / quality control is identified by scanning the calibrator / control barcodes, and the position of calibrator / quality control is assigned in the instrument system.
3. The sample to be tested is placed on the instrument sample rack chamber, and the corresponding test information is edited through the instrument supporting software.
4. Start the operation procedure, and all calibrator / quality control / sample processing steps will be automatically executed.

CALCULATION OF RESULTS

Calculation and interpretation of results will be performed automatically by software on BioCLIA instruments.

RESULT INTERPRETATION

Specimens with concentration <10 IU/mL, should be interpreted as negative;

Specimens with concentration ≥10 IU/mL, should be interpreted as positive.

The test results only reflect the amount of antigen specific IgG presented in the collected sample and should be diagnosed in conjunction with other laboratory and clinical findings.

CUT-OFF VALUE DETERMINATION

120 clinical samples, including 30 positive sera, 30 negative sera, 30 positive plasmas and 30 negative plasmas were collected and evaluated. Results were analyzed using the receiver-operating characteristic curve (ROC) and the cut-off value was determined at 10 IU/mL.

PERFORMANCE CHARACTERISTICS

ACCURACY / PIKED RECOVERY

This assay consists of two reference samples (low, high) which are traceable to WHO Standard Anti-ICA, CNS Code: 97/550. The reference materials are tested in triplicate to obtain a value of M, and calculated as: Measured deviation = (M - theoretical value)/ theoretical value x 100%*.

The accuracy/spiked recovery was determined by analyzing samples spiked with known amounts of anti-ICA antibodies into certain matrix. Anti-ICA antibody positive samples (low 5 IU/mL, mid 50 IU/mL, high 200 IU/mL) were spiked into two matrixes (20&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-ICA antibodies was calculated*.

	Matrix 20 IU/mL			Matrix 100 IU/mL		
Spiked Conc.	Obs	Exp.	Spiked Conc.	Obs	Exp.	Spiked Conc.
Neat	19.62		Neat	19.62		Neat
5 IU/mL	18.40	18.5	5 IU/mL	18.40	18.5	5 IU/mL
50 IU/mL	21.87	23.0	50 IU/mL	21.87	23.0	50 IU/mL
200 IU/mL	38.13	38.0	200 IU/mL	38.13	38.0	200 IU/mL

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

TRACEABILITY

Anti-ICA antibody concentration can be traced to WHO Standard Anti-ICA, CNS Code: 97/550.

PRECISION

A study based on guidance from (NCCLS) document EP-A[18] was performed.

Intra-assay precision: Four samples (negative, low, moderate, and high) were taken and tested with 10 replicates for each in a single run. Coefficient of variation (CV) was calculated for each of four samples. The results for intra-assay precision are shown in the table below.

Inter-assay precision: Four samples (negative, low, moderate, and high) were taken and tested with 4 replicates in a single run, two runs per day for 10 days. Coefficient of variation (CV) was calculated for each of four samples. The results for inter-assay precision are shown in the table below. *

Intra-assay precision: CV < 10%

Intra-Assay	Sample1	Sample2	Sample3	Sample4
Mean(IU/mL)	5.02	21.55	199.03	377.32
CV	5.0%	1.6%	6.4%	11.1%

Inter-assay precision: CV< 15%

Inter-Assay	Sample1	Sample2	Sample3	Sample4
Mean(IU/mL)	5.05	21.48	199.20	374.02
CV	5.7%	1.4%	6.3%	6.9%

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

LIMIT OF BLANK / DETECTION (LOB/LOD)

LOB/LOD was determined according to CLSI EP17-A guideline.

The assay is designed to have LoB/LoD of ≤ 0.5 IU/mL.

LINEARITY

The linear range of the assay is 2-400 IU/mL.

The linear range was determined by serially diluting a sample containing high levels of antigen specific IgG with a negative sample and covering 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 table below*:

Slope	Intercept	r
1.07	-0.50	0.99

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

INTERFERENCE

No interference has been observed with bilirubin, hemoglobin, triglycerides, rheumatoid factor (RF), human anti-mouse antibody (HAMA) at the levels 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 clinical sample results of the assay to the results of predicated assay. The results are shown in the table below. *

Clinical Sample		BioCLIA Autoimmune Reagent Kit		
		-	+	Total
Predicated Method	-	58	2	60
	+	2	38	40
	Total	60	40	100

Sensitivity	95.0%
Specificity	96.7%
Total agreement	96.0%

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 interpreted with all clinical and laboratory findings.

SYMBOLS

REF	Catalog Number		Use-by date
IVD	In Vitro diagnostic medical device	LOT	Lot Number
+2°C +8°C	Store between +2°C and +8°C		Consult Instruction for Use
	Manufacturer	EC REP	Authorized Representative in the European Community
CE	CE Marking		Contains Sufficient for<n>Tests
	Biological Risk		GHS07 Warning

BUFFER	Buffer
CONJ AP G	AP labeled anti-human IgG antibody
M ANTIGEN	Antigen coated microparticles
RCNS H_2O DIST	Reconstitute with distilled water before using

REFERENCES

1. Cooke A. An overview on possible mechanisms of destruction of the insulin-producing beta cell. Curr Top Microbiol Immunol 1989;164:125-42.
2. Harrison L, Campbell I, Colman P, Chosich N, Kay T, Tait B, et al. Type 1 diabetes: immunology and immunotherapy. Adv Endocrinol Metab 1990;1:35-94.
3. Bottazzo G, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. The Lancet 1974;304:1279-83.
4. Colman PG, Nayak RC, Campbell IL, Eisenbarth GS. Binding of cytoplasmic islet cell antibodies is blocked by human pancreatic glycolipid extracts. Diabetes 1988;37:645-52.
5. Riley W, Maclaren N. Islet-cell antibodies are seldom transient. The Lancet 1984;323:1351-52.
6. Soeldner JS, Tuttleman M, Srikanta S, Ganda OP, Eisenbarth GS. Insulin-dependent diabetes mellitus and autoimmunity: islet-cell autoantibodies, insulin autoantibodies, and beta-cell failure. N Engl J Med 1985;313:893-4.
7. Eisenbarth GS, Connelly J, Soeldner JS. The "natural" history of type I diabetes. Diabetes Metab Rev 1987;3:873–91.
8. Dean BM, Becker F, Mcnally JM, Tarn AC, Schwartz G, Gale EA, Bottazzo GF. Insulin autoantibodies in the pre-diabetic period: correlation with islet cell antibodies and development of diabetes. Diabetologia 1986;29:339-42.
9. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens. Jan 2001.
10. US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, Fourth Edition. Washington, DC: US Government Printing Office, May 1999.
11. World Health Organization. Laboratory Biosafety Manual. Geneva: World Health Organization. 2004.
12. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline - Third Edition. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.

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TECHNICAL ASSISTANCE

For technical assistance, contact your National Distributor.

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