BioCLIA® Autoimmune Reagent Kit

IA2

Chemiluminescent Microparticle Immunoassay

Magnetic bead chemiluminescence immunoassay (CLIA) for quantitative determination of anti-IA2 antibody in human serum

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
PRE BUF	Pre Buffer	Σ	Contains Sufficient for < *n* > Tests
CONJ AP G	Conjugate		Chemical Risk Warning
M STREP	Microparticle		Biological Risk Warning

HOB®

BioCLIA® Autoimmune Reagent Kit, IA2

Intended Use

BioCLIA IA2 assay is intended for the *in vitro* quantitative measurement of antibodies directed to IA2 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. This kit is be used on the instrument of BioCLIA 1200 and BioCLIA® 6500.

Catalog Numbers

MY00143 (50 Tests/kit)

MY00194 (100 Tests/kit)

Summary and Explanation

Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), results from a chronic autoimmune destruction of the insulin-secreting pancreatic beta cells, probably initiated by exposure of genetically susceptible host to an environmental agent. [1] Autoimmune destruction of beta cells is thought to be completely asymptomatic until 80 - 90% of the cells are lost. This process may take years to complete and may occur at any time. [2]

During the preclinical phase, this autoimmune process is marked by circulating autoantibodies to beta cell antigens. These autoantibodies are present years before the onset of type 1 diabetes and prior to clinical symptoms. Early studies utilized the immunofluorescence test for islet-cell antibodies (ICA), which has been difficult to standardize and is now replaced by a combination of several radioimmunoassays for antibodies against specific beta cell antigens, such is insulin (IAA), glutamic acid decarboxylase (IA2) and tyrosine phosphatase ICA 512 (IA2). [3]

IA2, a member of the protein tyrosine phosphatases family is localized in the dense granules of pancreatic beta cells and the second defined recombinant islet cell antigen. IA2 shares sequence identity with the islet cell antigen 512. The higher frequency of antibodies to IA2 is explained by the presence of autoantibodies directed to the COOH terminus of IA2 which is lacking in the ICA512 molecule. [4]

IA2 autoantibodies are present in the majority of individuals with new-onset type 1 diabetes and in individuals in the pre-diabetic phase of the disease. The appearance of autoantibodies to IA2 seems to be correlated with the rapid progression to overt type 1 diabetes. [5]

The combination of tests for IA265 and IA2 autoantibodies is highly relevant for risk assessment of type 1 diabetes in children and adolescence. The screening for IA265 and IA2 autoantibodies detect more than 90 % of subjects at risk for type 1 diabetes and may, therefore, possess the potential to replace ICA technique. [6]

Principles of the Procedure

BioCLIA IA2 assay is a two-step immunoassay using microparticle, enzyme-labeled chemiluminescent technology.

In the first step, the IA2 antigen coated magnetic microparticle, human serum and a pre buffer are mixed in a cuvette, which allows patient anti-IA2 antibody to bind. Secondly, after incubation, unbound reagent and sample matrix are removed by washing, and the microparticle -anti-IA2 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-IA2 IgG. According to a certain specific IgG RLU-concentration standard curve, the RLU tested can be interpreted to anti-IA2 IgG concentration in the sample expressed as IU/mL.

For quantitation of anti-IA2 antibodies, the BioCLIA IA2 assay utilizes a predefined lot specific main calibration curve that is uploaded into the instrument through the reagent cartridge barcode. The main calibration curve is created during manufacturing by using in-house standards that are traceable to the WHO standard material (NIBSC code: 97/550). Based on the main calibration 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 IA2 reagents are human serum. 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 specimens stored in refrigerator (2-8 °C) are valid for testing within 8 days. The unopened 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 opened/on board specimens should be tested within 10 hours. Three freeze-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 Standard on Bloodborne Pathogens. 7 Biosafety Level 2 8 or other appropriate biosafety practices 9, 10 should be used for materials that contain or possibly contain infectious agents.

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 kit reagents (IA2 Microparticle, Conjugate, Pre Buffer) at concentration between 0.0015% - 0.6%.

Storage Instructions

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 opened reagents or onboard reagents can be used up to 28 running days(2-8 °C). The lyophilized IA2 antigen coated microparticles is stable during 28 days after reconstitution when stored between 2- 8 °C. The BioCLIA® 1200 and BioCLIA® 6500 software monitors the expiration of the reagent cartridge. The system will not accept expired reagents. Three freeze-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.

- **IA2 Microparticle** 1 or 2 bottle(s) (1 X sq 4 mL) lyophilized IA2 antigen coated microparticles. Dilute with 4ml distilled water per bottle before using.

M	IA2	RCNS	H_2O	DIST

Preservatives: 0.0015% < Proclin 300 < 0.6%.

- **Conjugate** One bottle (6.75/13.5 mL) AP labeled Anti-human IgG in 0.05 M MES (pH6.0) Buffer.

CONJ	AP	G

Preservatives: 0.0015% < Proclin 300 < 0.6%.

- **Pre Buffer** One bottle (2.5/5 mL) 0.05 M Tris (pH7.4) Buffer.

PRE	BUF

Preservatives: 0.0015% < Proclin 300 < 0.6%.

Kit Component Supplied Separately

Additional Materials Required But Not Provided:

- BioCLIA® 1200 (Cat No. MA00139)
- BioCLIA®6500 (Cat No. MA00243)
- BioCLIA Autoimmune Calibrator Set, IA2 (Cat No. MY00245, 2 x 1 mL; Cat No. MY00296, 4 x 1 mL)
- BioCLIA Autoimmune Control Set, IA2 (Cat No. MY00347, 2 x 1 mL; Cat No. MY00398, 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 for preparation, setup, dilutions, adjustment, assay and quality control procedures.

Reconstitute the lyophilized IA2 antigen coated microparticles with distilled water (4ml distilled water per bottle). Blend for more than 30 minutes in low speed, transfer the solution to the supplied empty Microparticle bottle. For the kit with 100 tests, user should transfer both bottles of reconstitute antigen-microparticle solution to supplied empty microparticle bottle before using.

Users should have the periodic calibration procedure for every 28 running days from last calibration. Besides, a calibration procedure should be carried out when a new batch of BioCLIA IA2 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 build in master calibration curve and specified two-point calibrator set for instrument, the BioCLIA will automatically calculate the anti-IA2 antibodies concentration of each specimen and interpret the results into IU/mL. The concentration of anti-IA2 antibody sample concentration is reported as < 2 IU/mL when it is lower than the minimum detection limit, while reported as > 4000 IU/mL when it is higher than the range of measurement.

Sample Dilution

The specimens are diluted with 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 60 positive sera and 60 negative sera, were collected and valued. These samples were venous blood from human the aged between 0 - 80, sealed and stored at 2 - 8 oC. Results of 120 clinical samples tested by the BioCLIA IA2 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 identify.

ACCURACY

This assay consists of two reference samples (low, high) which are traceable to WHO Standard Anti-IA2, 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%.*

Samples (IU/mL)	Ave. Conc.	Exp.	Measured Deviation
Sample 1 (low)	5.07	5	1.4%
Sample 2 (high)	398.77	400	-0.3%

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

TRACEABILITY

Anti-IA2 antibody concentration can be traced to WHO Standard Anti-IA2, CNS Code: 97/550.

PRECISION

A study based on guidance from CLSI document EP5-A2 was performed for determining the precision of BioCLIA IA2 kit precision. Human serum in the in-house reference panel (low, mid, high) was tested with 10 replicates for intra-assay precision evaluation, while in 4 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 runs per 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)	5.14	49.34	1510.50	3576.3
CV	3.7%	2.5%	2.2%	7.5%

Inter-assay precision: CV ＜ 15%

Inter-Assay	RP1	RP2	RP3	RP4
Mean(IU/ml)	5.14	49.85	1510.88	3525
CV	4.3%	2.3%	1.9%	8.1%

*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 IA2 assay was lower than 0.7 IU/mL, which is below the analytical measuring range of the assay.

ASSAY REPORTABLE RANGE

The BioCLIA IA2 kit has a reportable linear range of 2- 4000 IU/mL. The linear range was determined by diluting a high positive anti-IA2 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
0.99	-0.12	1.00

Assay linear range is 2-4000 IU/mL. Results below the lower limit will be reported as < 2 IU/mL, while those are above the upper limit will be reported as > 4000 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 IA2 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 IA2 assay to the predicated assay.

Clinical Sample		BioCLIA IA2		
		-	+	Total
Predicated Method	-	58	2	60
	+	2	38	40
	Total	60	40	100

Sensitivity	95.0%
Specificity	96.7%
Total agreement	96%

Limitations

- The effectiveness of this kit is only confirmed for human serum, 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.

References

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Technical Assistance

For technical assistance, contact your National Distributor.

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