BioCLIA® Autoimmune Reagent Kit

β2 Glycoprotein 1 IgM

Chemiluminescent Microparticle Immunoassay

Magnetic bead chemiluminescence immunoassay (CLIA) for semi-quantitative determination of anti-β 2 Glycoprotein 1 IgM 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		Contains Sufficient for < *n* > Tests
CONJ AP M	Conjugate		Chemical Risk Warning
M STREP	Microparticle		Biological Risk Warning

BioCLIA® Autoimmune Reagent Kit, β2 Glycoprotein 1 IgM

Intended Use

BioCLIA β2 Glycoprotein 1 IgM assay is intended for the in vitro semi-quantitative measurement of anti- β2 Glycoprotein 1 IgM antibodies in human serum and plasma as an aid in the diagnosis of primary Antiphospholipid Syndrome (APS) and those secondary to systemic lupus erythematosus (SLE) or SLE-like disorders in conjunction with other laboratory and clinical findings. This kit is used on the instrument of BioCLIA® 1200 and BioCLIA® 6500.

Catalog Numbers

MY00126 (50 Tests/kit)
MY00177 (100 Tests/kit)

Summary and Explanation

Antibodies against β2-glycoprotein I belong to the group of anti-phospholipid antibodies mainly targeted against complexes composed of negatively charged phospholipids (e.g cardiolipin) and plasma proteins like β2- glycoprotein I, prothrombin, protein C or protein S. [1, 2] Reactivity against isolated β2-glycoprotein I can also be found.

β2-glycoprotein I, also called apolipoprotein H, is a 50 kDa β2 globuline which is associated in vivo with lipoprotein, platelets and phospholipids and which seems to inhibit the intrinsic coagulation pathway, the prothrombinase activity and the ADP-dependent platelet aggregation.

Anti-phospholipid antibodies are frequently found in sera of patients with systemic lupus erythematosus and related diseases and are typical for the secondary development of an antiphospholipid syndrome (APS). Whilst, anti-phospholipid antibodies in patients with no other autoimmune diseases characterize a primary APS.

About 30-60% anti-phospholipid syndrome (APS) patients are anti-β-2 glycoprotein I antibodies positive.[3] They are also closely associated with thrombosis. Determination of anti-β-2 glycoprotein I antibodies can significantly increase the prediction rate of thrombosis complications. [4] As these antibodies only appear in autoimmune disease patients, they are regarded as autoimmune thrombus markers to distinguish autoimmune diseases from infectious diseases. [5] Anti-β-2 glycoprotein I antibodies have a specificity of 98% while anti-cardiolipin antibodies (aCL) of 75% for APS diagnosis; however, the sensitivity is only 54% which is lower than the aCL. Besides, concentration of anti-β2 glycoprotein I antibodies is related to the severity of thrombosis in systemic lupus erythematosus (SLE) patients. [6]

Principles of the Procedure

BioCLIA β2 Glycoprotein 1 IgM assay is a two-step immunoassay using microparticle, enzyme-labeled chemiluminescent technology.

In the first step, the streptavidin coated magnetic Microparticle, the biotinylated β2 Glycoprotein 1 antigen and human serum/plasma sample are mixed in an assay tube, which allows patient specific anti-β2 Glycoprotein 1 IgM to bind. Secondly, after incubation, unbound biotinylated β2 Glycoprotein 1 and sample matrix are removed by washing, and the Microparticle-β2 Glycoprotein 1 antigen-anti-β2 Glycoprotein 1 IgM antibodies immune complexes are kept with the help of a magnetic separator. Third, anti-human IgM 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-β2 Glycoprotein 1 IgM. According to the anti-β2 Glycoprotein 1 IgM RLU-concentration standard curve, the RLU tested can be interpreted to anti-β2 Glycoprotein 1 IgM concentration in the sample expressed as RU/mL.

For semi-quantitation of anti-β2 Glycoprotein 1 IgM antibodies, the BioCLIA β2 Glycoprotein 1 IgM assay utilizes a predefined lot specific Master Curve that is uploaded into the instrument through the reagent Master Calibration Curve barcode. Based on the Master Curve, and results obtained by running two Calibrators, an instrument specific Working Curve is created, which is used to calculate anti-β 2 Glycoprotein 1 IgM antibodies concentration RU/mL from the relative luminescent units (RLU) obtained for each sample.

Specimen Collection

The appropriate specimen types for BioCLIA β2 Glycoprotein 1 IgM Reagents 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 specimens stored 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 tested within 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 Standard on Bloodborne Pathogens. [7] Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or possibly contain infectious agents. [8, 9, 10] Avoid contacting 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 (β 2 Glycoprotein 1 IgM Antigen, Conjugate) 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 56 consecutive days (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 (at -20°C) -thaw 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.

- **β2 Glycoprotein 1 IgM Antigen** 1 bottle (2.5/5 mL) Biotinylated β2GP1 antigen in 0.01 M PBS (pH7.4) buffer with stabilizer. β2GP1-M | BIOT

  Preservatives: 0.0015% < Proclin 300 < 0.6%.

- **Conjugate** 1 bottle (6.75/13.5 mL) AP labeled anti-human IgM antibodies in 0.05 M MES (pH6.0) Buffer with stabilizer. CONJ | AP | M

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

- **Microparticle** 1 bottle (2.5/5 mL) Streptavidin-microparticles in 0.01 M 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, β2 Glycoprotein 1 IgM
  (Cat No. MY00228, 2 x 1 mL;
  Cat No. MY00279, 4 x 1 mL)
- BioCLIA Autoimmune Control Set, β2 Glycoprotein 1 IgM
  (Cat No. MY00330, 2 x 1 mL;
  Cat No. MY00381, 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.

Users should have the periodic calibration procedure for every 56 consecutive days from last calibration. Besides, a calibration procedure should be carried out when a new batch of BioCLIA β 2 Glycoprotein 1 IgM 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 specified two-point calibrator set for the instrument, the BioCLIA will automatically calculate the auto-antibodies concentration of each specimen and interpret the results into RU/mL. The concentration of anti-β2 Glycoprotein 1 IgM antibody sample is reported as < 2 RU/mL when it is lower than the minimum detection limit, while reported as > 400 RU/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 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 β 2 Glycoprotein 1 IgM kit were analyzed using the receiver-operating characteristic curve (ROC) and the cut-off value was determined at 20 RU/mL.

Test Result Interpretation

Specimen with concentration < 20 RU/mL, interpreted as negative;

Specimen with concentration ≥ 20 RU/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-β 2 Glycoprotein 1 IgM antibodies into certain matrix.Anti-β 2 Glycoprotein 1 IgM antibody positive samples (low 100 RU/mL, mid 200 RU/mL, high 300 RU/mL) were spiked into two matrixes (50 & 100 RU/mL) separately at the volume ratio of 1:9, making totally 6 spiked samples and each sample was tested in triplicate. The spiked recovery for the concentration of anti-β2 Glycoprotein 1 IgM antibodies was calculatd.*

	Matrix 50 RU/mL			Matrix 100 RU/mL		
Spiked Conc.	**Obs**	**Exp.**	**Obs/Exp**	**Obs**	**Exp.**	**Obs/Exp**
Neat	48.01			94.43		
100 RU/mL	53.09	53.2	99.8%	94.49	95.0	99.5%
200 RU/mL	64.60	63.2	102.2%	104.22	105.0	99.3%
300 RU/mL	75.35	73.2	102.9%	111.07	115.0	96.6%

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

TRACEABILITY

The reported values were determined with multiple runs on the BioCLIA® 1200 and BioCLIA® 6500 using specific reagents against an in-house standard. Results are reported in RU/mL, which is interpreted from relative light unit (RLU). Method comparison test showed good sensitivity and specificity.

PRECISION

A study based on guidance from CLSI document EP5-A2 was performed for determining the precision of BioCLIA β2 Glycoprotein 1 IgM 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 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(RU/ml)	9.77	19.78	100.91	358.26
CV	4.92%	3.82%	5.25%	4.18%

Inter-assay precision: CV < 15%

Inter-Assay	RP1	RP2	RP3	RP4
Mean(RU/ml)	9.97	19.89	99.73	346.97
CV	7.99%	5.69%	7.03%	6.61%

*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 β 2 Glycoprotein 1 IgM assay was lower than 1.0 RU/mL, which is below the analytical measuring range of the assay.

ASSAY REPORTABLE RANGE

The BioCLIA β 2 Glycoprotein 1 IgM kit has a reportable linear range of 2 - 400 RU/mL. The linear range was determined by diluting a high positive anti-β 2 Glycoprotein 1 IgM 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
0.98	-0.40	0.99

Assay linear range is 2-400 RU/mL. Results below the lower limit will be reported as < 2 RU/mL, while those are above the upper limit will be reported as > 400 RU/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 β 2 Glycoprotein 1 IgM assay performances 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 β2 Glycoprotein 1 IgM assay to the predicated assay.

Clinical Sample		BioCLIA β 2 Glycoprotein 1 IgM		
		-	+	Total
Predicated Method	-	57	3	60
	+	4	36	40
	Total	61	39	100

Sensitivity	90.0%
Specificity	95.0%
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 integrate with all clinical and laboratory findings.

References

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Technical Assistance

For technical assistance, contact your National Distributor.

17th April 2019

Revision 10