BioCLIA Autoimmune Reagent Kit, β2 Glycoprotein 1 IgG

(Chemiluminescent Microparticle Immunoassay)

Kit size	Cat. No.	GTIN Code
50 Tests/kit	MY00125	06924030402402
100 Tests/kit	MY00176	06924030402914

INTENDED USE

The BioCLIA Autoimmune Reagent Kit, β2 Glycoprotein 1 IgG assay is intended for the in vitro semi-quantitative measurement of anti- β2 Glycoprotein 1 IgG 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. It is an *in vitro* diagnostic medical device intended for laboratory professional use.

SUMMARY AND EXPLANATION OF THE TEST

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 Autoimmune Reagent Kit is a two-step immunoassay. The biotinylated antigen of interest is mixed with streptavidin coated magnetic microparticles. Specific IgG in the patient's serum/plasma reacts with these to form a IgG-antigen complex. After incubation, a washing step removes the free and non-specifically bound molecules. Subsequently enzyme labeled anti-human IgG antibodies (conjugate) is added and this binds to the IgG-antigen complex. After further incubation, a second washing step removes the unbound conjugate. 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%~0.6% 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 5-Bromo-5-Nitro-1, 3-Dioxane (BND) < 1% as preservative 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 Country in which the user and/or the patient

is established.

MATERIALS SUPPLIED

• **Antigen**   Biotinylated antigen in PBS (pH 7.0-7.4).

| ANTIGEN | BIOT |

Preservatives: 0.0015%＜ProClin 300 < 0.6%.

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

| CONJ | AP | G |

Preservatives: 0.0015%＜ProClin 300 < 0.6%.

• **Microparticle**   Streptavidin coated microparticles in 0.01M PBS (pH 7.4).

| M | STREP |

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

Components	50 Tests/Kit	100 Tests/Kit
Antigen	1 x 2.5 mL	1 x 5 mL
Conjugate	1 x 6.75 mL	1 x 13.5 mL
Microparticle	1 x 2.5 mL	1 x 5 mL

ADDITIONAL MATERIALS SUPPLIED SEPARATELY

Product	CATALOGUE No.
BioCLIA Autoimmune Calibrator Set, β2 Glycoprotein 1 IgG	MY00227 (2 x 1 mL) MY00278(4 x 1 mL)
BioCLIA Autoimmune Control Set, β2 Glycoprotein 1 IgG	MY00329 (2 x 1 mL) MY00380 (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.

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 reagents, 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 met.

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 <20 RU/mL, should be interpreted as negative;

Specimens with concentration ≥20 RU/mL, should be interpreted as positive.

The test results only reflect the amount of antigen specific IgG present in the 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 20 RU/mL.

PERFORMANCE CHARACTERISTICS

ACCURACY / SPIKED RECOVERY

The accuracy/spiked recovery was determined by analyzing samples spiked with known amounts of antibody into sample matrix. Specific antibody positive samples (low 100 RU/mL, mid 200 RU/mL, high 300RU/mL) were spiked into two matrixes (50 and 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 autoantibodies to specific antigen was calculated. *

Spiked Conc.	Matrix 50 RU/mL			Matrix 100 RU/mL		
	Obs	Exp.	Obs/Exp	Obs	Exp.	Obs/Exp
Neat	47.54			98.07		
100 RU/mL	53.74	52.8	101.8%	102.67	98.3	104.5%
200 RU/mL	66.28	62.8	105.6%	108.42	108.3	100.2%
300 RU/mL	75.62	72.8	103.9%	117.94	118.3	99.7%

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

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(RU/mL)	9.78	20.28	99.19	350.56
CV	6.11%	5.24%	4.83%	4.52%

Inter-assay precision: CV< 15%				
Inter-Assay	Sample1	Sample2	Sample3	Sample4
Mean(RU/mL)	9.93	19.76	99.99	350.51
CV	8.84%	6.19%	6.77%	6.98%

*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 RU/mL.

LINEARITY

The linear range of the assay is 2-400 RU/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
0.97	-0.35	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 $\leqslant$ 150 mg/dL;
- Triglycerides $\leqslant$ 1,000 mg/dL;
- Rheumatoid factor (RF) $\leqslant$ 1,000 IU/mL;
- Human anti-mouse antibody (HAMA) $\leqslant$ 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, β2 Glycoprotein 1 IgG		
		-	+	Total
Predicated Method	-	62	3	65
	+	3	32	35
	Total	65	35	100

Sensitivity	91.4%
Specificity	95.4%
Total agreement	94.0%

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	Manufacturer	EC REP	Authorized Representative in the European Community
CE	CE Marking	Σ	Contains Sufficient for<n>Tests
Biological Risk	Biological Risk	!	GHS07 Warning

ANTIGEN BIOT	Biotinylated antigen
CONJ AP G	AP labeled anti-human IgG antibody
M STREP	Streptavidin coated microparticles

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TECHNICAL ASSISTANCE

For technical assistance, contact your National Distributor.

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