BioCLIA Autoimmune Reagent Kit, Ro52

(Chemiluminescent Microparticle Immunoassay)

Kit size	Cat. No.	GTIN Code
50 Tests/kit	MY00104	06924030402198
100 Tests/kit	MY00155	06924030402709

INTENDED USE

The BioCLIA Autoimmune Reagent Kit, Ro52 assay is intended for the *in vitro* semi-quantitative measurement of IgG antibodies directed to Ro52 in human serum and plasma as an aid in the diagnosis of anti-nuclear antibody (ANA) diseases 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

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), systemic lupus erythematosus (SLE), Sjögren's syndrome, progressive systemic sclerosis, polymyositis/dermatomyositis, overlap syndrome, and limited types of progressive systemic sclerosis (CREST syndrome).

Ro52 is a 52-kDa protein detected in serum of patients with Sjogren's syndrome, systemic lupus erythematosus (SLE), dermatomyositis, without any defined specificity. It belongs to the TRIM protein family (TRIM21) with different functional structure domain like RING finger, B-box or curly spiral. [2, 3] The effect of Ro52 on immune defense is increasingly concerned, such as the interaction between Ro52 and IgG molecules, or how the Ro52 becomes an E3 ubiquitin ligase and act on signal molecules. So far, the pathogenic mechanism of Ro52 in autoimmune diseases is not clear. Ro52 proteins are confirmed to be associated with SS-A/Ro complex function. [4]

Due to the appearance of anti-Ro52 antibodies in a variety of autoimmune diseases, it should not be taken as a specific indicator of SLE or Sjögren's syndrome but should be combined with other clinical findings.

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** Lyophilized biotinylated antigen.

ANTIGEN	BIOT
RCNS	H_2O	DIST

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 sq 4 mL*	2 x sq 4 mL*
Conjugate	1 x 6.75 mL	1 x 13.5 mL
Microparticle	1 x 2.5 mL	1 x 5 mL

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

ADDITIONAL MATERIALS SUPPLIED SEPARATELY

Product	**CATALOGUE No.**
BioCLIA Autoimmune Calibrator Set, CTD Screen	MY00970 (2 x 1 mL) MY00981 (4 x 1 mL)
BioCLIA Autoimmune Control Set, CTD Screen	MY00971 (2 x 1 mL) MY00982 (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 Biotinylated Antigen Reconstitution

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

	Matrix 50 RU/mL			Matrix 100 RU/mL		
Spiked Conc.	Obs	Exp.	Obs/Exp	Obs	Exp.	Obs/Exp
Neat	47.57			102.58		
100 RU/mL	55.27	52.8	104.6%	91.95	102.3	89.9%
200 RU/mL	66.68	62.8	106.2%	105.89	112.3	94.3%
300 RU/mL	78.08	72.8	107.2%	130.62	122.3	106.8%

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

TRACEABILITY

This assay system is calibrated in relative arbitrary units since no international reference preparation is available for this assay. The reported values were determined with multiple runs on the BioCLIA 6500 and BioCLIA 500 using specific reagents against an in-house standard.

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)	9.81	19.75	97.50	357.29
CV	0.7%	2.3%	1.3%	1.24%

Inter-assay precision: CV< 15%

Inter-Assay	Sample1	Sample2	Sample3	Sample4
Mean(RU/mL)	9.73	20.27	103.04	353.55
CV	6.0%	5.4%	5.9%	2.9%

*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 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
0.93	+0.37	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, Ro52		
		-	+	Total
Predicated Method	-	50	0	50
	+	0	50	50
	Total	50	50	100

Sensitivity	100.0%
Specificity	100.0%
Total agreement	100.0%

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

ANTIGEN BIOT	Biotinylated antigen
CONJ AP G	AP labeled anti-human IgG antibody
M STREP	Streptavidin coated microparticles
RCNS H_2O DIST	Reconstitute with distilled water before using

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

REFERENCES

1. Prince HE, Hogrefe WR. Evaluation of a line immunoblot assay for detection of antibodies recognizing extractable nuclear antigens. Journal of Clinical Laboratory Analysis 1998;12:320-4.
2. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. Diagnostic Pathology 2009;4:1-10.
3. Nakamura M, Kondo H, Mori T, Komori A, Matsuyama M, Ito M, et al. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis †. Hepatology 2007;45:118-27.
4. Lock RJ, Unsworth DJ. Antibodies to extractable nuclear antigens. Has technological drift affected clinical interpretation? Journal of Clinical Pathology 2001;54:187-90.
5. Hernández-Molina G, Leal-Alegre G, Michel-Peregrina M. The meaning of anti-Ro and anti-La antibodies in primary Sjögren's syndrome. Autoimmunity Reviews 2011;10:123-5.
6. Stahnke G, Meier E, Scanarini M, Northemann W. Eukaryotic Expression of Recombinant Human Centromere Autoantigen and its use in a Novel ELISA for Diagnosis of CREST Syndrome. Journal of Autoimmunity 1994;7:107-18.
7. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens. Jan 2001.
8. US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, Fourth Edition. Washington, DC: US Government Printing Office, May 1999.
9. World Health Organization. Laboratory Biosafety Manual. Geneva: World Health Organization. 2004.
10. 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.
11. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. Diagnostic Pathology 2009;4:1-10.

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The eIFU is available on Website:

http://en.hob-biotech.com/usercenter/login.aspx

TECHNICAL ASSISTANCE

For technical assistance, contact your National Distributor.

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