# BioCLIA® Autoimmune Reagent Kit

## *Jo-1*

**Chemiluminescent Microparticle Immunoassay**

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

# BioCLIA® Autoimmune Reagent Kit, Jo-1

**Intended Use**

BioCLIA Jo-1 assay is is intended for the *in vitro* semi-quantitative measurement of IgG antibodies directed to Jo-1 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. This kit is used on the instrument of BioCLIA® 1200 and BioCLIA® 6500.

**Catalog Numbers**

MY00102 (50 Tests/kit)
MY00153 (100 Tests/kit)

**Summary and Explanation**

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).

Jo-1 is a 50 kDa cytoplasm phosphoprotein, the histidyl-tRNA synthetase. It plays an important role in transporting free histidine to the corresponding tRNA and translating peptide from mRNA templates. [2]
Anti-Jo-1 antibodies are commonly seen in polymyositis (PM) patients' sera with a sensitivity of 40% to 50%, 25% in PM/DM patients, less than 10% in DM (dermatomyositis) patients, and not detected in other autoimmune diseases. [3]

**Principles of the Procedure**

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

In the first step, the streptavidin coated magnetic microparticle, the biotinylated Jo-1 and human serum/plasma sample are mixed in an assay tube, which allows patient specific anti-Jo-1 to bind. Secondly, after incubation, unbound biotinylated Jo-1 and sample matrix are removed by washing, and the microparticle-Jo-1-anti-Jo-1 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-Jo-1 specific IgG. According to the anti-Jo-1 specific IgG RLU-concentration standard curve, the RLU tested can be interpreted to anti-Jo-1 specific IgG concentration in the sample expressed as RU/mL.

For semi-quantitation of anti-Jo-1 antibodies, the BioCLIA Jo-1 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-Jo-1 antibodies concentration RU/mL from the relative luminescent units (RLU) obtained for each sample.

**Specimen Collection**

The appropriate specimen types for BioCLIA Jo-1 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. [5] Biosafety Level 2 [6] or other appropriate biosafety practices should be used for materials that contain or possibly contain infectious agents. [7, 8] 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 (Jo-1 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 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.

BioCLIA Jo-1

- **Jo-1 Antigen** 1 bottle (2.5/5 mL) Biotinylated Jo-1 antigen in PBS (pH7.0-7.4) buffer with stabilizer.

Preservatives: NaN3 < 0.1% Jo-1 | BIOT

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

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, Jo-1 (Cat No. MY00204, 2 x 1 mL; Cat No. MY00255, 4 x 1 mL)
- BioCLIA Autoimmune Control Set, Jo-1 (Cat No. MY00306, 2 x 1 mL; Cat No. MY00357, 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 Jo-1 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-Jo-1 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 oC. Results of 120 clinical samples tested by the BioCLIA Jo-1 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-Jo-1 antibodies into certain matrix. Anti-Jo-1 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-Jo-1 antibodies was calculatd.*

| | Matrix 50 RU/mL | | | Matrix 100 RU/mL | | |
|---|---|---|---|---|---|---|
| **Spiked Conc.** | **Obs** | **Exp.** | **Obs/Exp** | **Obs** | **Exp.** | **Obs/Exp** |
| **Neat** | 39.29 | | | 67.61 | | |
| **100 RU/mL** | 43.72 | 45.4 | 96.4% | 66.78 | 70.8 | 94.3% |
| **200 RU/mL** | 49.41 | 55.4 | 89.2% | 74.66 | 80.8 | 92.3% |
| **300 RU/mL** | 60.37 | 65.4 | 92.4% | 82.45 | 90.8 | 90.8% |

*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 Jo-1 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) | 9.97 | 19.87 | 98.39 | 359.16 |
| CV | 0.9% | 0.4% | 0.3% | 1.4% |

Inter-assay precision: CV < 15%

| Inter-Assay | RP1 | RP2 | RP3 | RP4 |
|---|---|---|---|---|
| Mean(RU/ml) | 9.40 | 20.04 | 96.66 | 352.14 |
| CV | 2.3% | 3.5% | 6.2% | 3.1% |

*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 Jo-1 assay was lower than 1.0 RU/mL, which is below the analytical measuring range of the assay.

ASSAY REPORTABLE RANGE

The BioCLIA Jo-1 kit has a reportable linear range of 2 - 400 RU/mL. The linear range was determined by diluting a high positive anti-Jo-1 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 |
|---|---|---|
| 1.02 | -0.19 | 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 Jo-1 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 Jo-1 assay to the predicated assay.

| Clinical Sample | | BioCLIA Jo-1 | | |
|---|---|---|---|---|
| | | - | + | Total |
| Predicated Method | - | 71 | 0 | 71 |
| | + | 0 | 29 | 29 |
| | Total | 71 | 29 | 100 |

| | |
|---|---|
| Sensitivity | 100.0% |
| Specificity | 100.0% |
| Total agreement | 100.0% |

STABILITY

The BioCLIA assay is designed to have a kit stress stability, freeze-thaw stability, onboard stability, open vial stability, real time stability all with signal retention rate above 70% within testing period. Transportation stability should with signal retention rate above 85% within testing period.

**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 integrated with all clinical and laboratory findings.

**References**

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For technical assistance, contact your National Distributor.

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