



MAGLUMI® ENA Screen (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the semi-quantitative determination of IgG class antibodies to extractable nuclear antigens (ENA) in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis of individuals with suspected or confirmed multiple systemic autoimmune diseases (Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MCTD), Sjögren's Syndrome (SS), Systemic Sclerosis (SSc) and Polymyositis/Dermatomyositis (PM/DM)).

SUMMARY

Antinuclear antibodies (ANA) are a diverse group of autoantibodies that recognize multiple intracellular antigens, classically consisting of nuclear specificities such as deoxyribonucleic acid or small nuclear ribonucleoproteins¹. It is currently accepted that ANA contain two major types of antibodies, the first group includes antibodies against DNA and histones and the second group includes autoantibodies to extractable nuclear antigens (ENA)²⁻⁴. Antigens that are extracted from saline extracts of nuclei are known as ENA (e.g., Sm/RNP, Sm, SS-A/Ro, SS-B, Jo-1, Scl-70)^{3,4}. These nuclear particles recognized by ENA antibodies have essential intracellular functions such as replication and transcription, and thus are structurally conserved among species⁵. Testing for autoantibodies to ENA is often helpful to confirm the diagnosis of a specific connective tissue disease^{3,6}. In particular, Sm/RNP antibodies are associated with Mixed Connective Tissue Disease (MCTD), Sm antibodies with Systemic Lupus Erythematosus (SLE), SS-A/Ro and SS-B antibodies with Sjögren's Syndrome (SS), Jo-1 antibodies with Polymyositis/Dermatomyositis (PM/DM), Scl-70 antibodies with Systemic Sclerosis (SSc)³⁻⁶. Testing for ENA autoantibodies may be considered if the ANA is positive or if there are clinical features suggestive of one of the aforementioned autoimmune diseases^{3,6}. However, the significance of any positive test result for anti-ENA antibodies in the absence of a positive ANA test must be interpreted with caution⁶.

TEST PRINCIPLE

Indirect chemiluminescence immunoassay.

The prediluted sample, buffer, magnetic microbeads coated with ENA (Sm/RNP, Sm, SS-A/Ro, SS-B, Jo-1, Scl-70) mixed thoroughly and incubated to form immune-complexes. After incubation, materials bound to the magnetic microbeads are held in a magnetic field while unbound materials are washed away during a wash cycle. Then adding ABEI labeled with mouse monoclonal anti-human IgG antibody, incubated to form sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then another wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of anti-ENA antibodies present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Lyophilized Magnetic Microbeads	Magnetic microbeads coated with ENA (Sm/RNP, Sm, SS-A/Ro, SS-B, Jo-1, Scl-70) (~16.8 µg/bottle) in PBS buffer, NaNa ₃ (<0.1%).	1 bottle	1 bottle	1 bottle
Magnetic Microbeads Buffer	PBS buffer, NaNa ₃ (<0.1%).	2.8 mL	2.8 mL	2.8 mL
Calibrator Low	A low concentration of anti-ENA antibodies in PBS buffer, NaNa ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of anti-ENA antibodies in PBS buffer, NaNa ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Buffer	BSA, NaNa ₃ (<0.1%).	13.5 mL	7.5 mL	4.8 mL
ABEI Label	ABEI labeled with anti-human IgG monoclonal antibody (mouse) (~25.0 ng/mL) in Tris-HCl buffer, NaNa ₃ (<0.1%).	23.5 mL	12.5 mL	7.8 mL
Diluent	PBS buffer, NaNa ₃ (<0.1%).	25.0 mL	15.0 mL	8.0 mL
Control 1	A low concentration of anti-ENA antibodies (10.0 AU/mL) in PBS buffer, NaNa ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Control 2	A high concentration of anti-ENA antibodies (50.0 AU/mL) in PBS buffer, NaNa ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Magnetic Microbeads is lyophilized and must be reconstituted with Magnetic Microbeads Buffer, refer to the Preparation of Magnetic Microbeads section.				

The control barcode labels are provided.

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Lyophilized Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads reconstitution and mixing instructions, refer to the Preparation of Magnetic Microbeads and Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date



0123



130217504M: 100 tests/kit

130617504M: 50 tests/kit

130717504M: 30 tests/kit

Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 18-25°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Serum	Tubes without additive/accessory, or tubes containing clot activator or clot activator with gel.
Plasma	K2-EDTA or sodium heparin tubes

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use heat-inactivated samples or grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 20 µL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 8 hours at 18-25°C, or 7 days at 2-8°C, or 3 months frozen at -20°C or colder. Frozen specimens subjected to up to 3 freeze/thaw cycles have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, anti-ENA antibodies concentrations above the analytical measuring interval, can be diluted with Diluent either automated dilution protocol or manual dilution procedure. The recommended dilution ratio is 1:20. The concentration of the diluted sample must be >10 AU/mL.
- For manual dilution, multiply the result by the dilution factor. For dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

PROCEDURE

Materials Provided

ENA Screen (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3, MAGLUMI X6, or Integrated System Biolumi 8000 and Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.

Preparation of Magnetic Microbeads <i>The Magnetic Microbeads is provided in a lyophilized form. The vial containing the lyophilized magnetic microbeads must be opened carefully and reconstituted with the Magnetic Microbeads Buffer.</i> <i>Remove 2 mL Magnetic Microbeads Buffer from the magnetic microbeads tube (blue collar and serrated reagent tube on the bottom) into the vial containing lyophilized magnetic microbeads before use, cover with a rubber stopper, and gently shake. Allow the dissolved magnetic microbeads to stand for 10-15 minutes.</i> <i>Swirl gently to ensure homogeneity. Avoid heavy shaking when dissolving (avoid formation of foam).</i> <i>Transfer all reconstituted magnetic microbeads in vial to the magnetic microbeads tube and mix it with the remaining Magnetic Microbeads Buffer evenly, then place prepared kit onto the analyzer.</i> <i>After use, the kit including the reconstituted magnetic microbeads should be stored at 2-8°C in an upright position.</i>

- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the Snibe internal reference standard.
Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.
Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 7 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.
- Each time a new kit is used.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines⁷.

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the ENA Screen assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi system and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order ENA Screen (CLIA) Controls (REF: 160201406MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the anti-ENA antibodies concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in AU/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

The optimal cut-off of the ENA Screen assay was obtained by testing 153 confirmed systemic autoimmune diseases (such as Systemic Lupus Erythematosus, Mixed Connective Tissue Disease, Sjögren's Syndrome, Systemic Sclerosis and Polymyositis/Dermatomyositis), 63 patients with other disease and 253 apparently healthy individuals.

- Samples with anti-ENA antibodies concentration <20.0 AU/mL should be considered negative.
- Samples with anti-ENA antibodies concentration ≥20.0 AU/mL should be considered positive.

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the anti-ENA antibodies results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies^{8, 9}. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed¹⁰.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (AU/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV
Serum Pool 1	5.027	0.195	3.88	0.094	1.87	0.312	6.21
Serum Pool 2	21.403	0.713	3.33	0.424	1.98	0.993	4.64
Serum Pool 3	98.019	2.826	2.88	1.708	1.74	4.543	4.63
Plasma Pool 1	4.938	0.193	3.91	0.102	2.07	0.278	5.63
Plasma Pool 2	20.763	0.720	3.47	0.136	0.66	0.920	4.91
Plasma Pool 3	100.488	2.835	2.82	2.275	2.26	3.966	3.95
Control 1	9.943	0.348	3.50	0.212	2.13	0.556	5.59
Control 2	49.712	1.745	3.51	0.945	1.90	2.390	4.81

Linear Range

1.00-200 AU/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

0.850-4000 AU/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.500 AU/mL.

Limit of Detection (LoD) =0.850 AU/mL.

Limit of Quantitation (LoQ) =1.00 AU/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Bilirubin	40 mg/dL	Rheumatoid factor	500 IU/mL
Hemoglobin	1000 mg/dL	HAMA	40 ng/mL
Intralipid	2000 mg/dL		

Cross-Reactivity

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactants in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Cross-reactant	No interference up to	Cross-reactant	No interference up to
anti-CCP	500 U/mL	anti-Rib-P IgG	400 AU/mL
anti-dsDNA IgG	800 IU/mL	anti-Centromeres IgG	400 AU/mL
anti-Histones IgG	400 AU/mL		

Clinical Sensitivity

The clinical sensitivity was determined for a panel of 189 systemic autoimmune diseases specimens. The clinical sensitivity was calculated to be 67.7%. The following results were obtained:

Specimen Category	ENA Screen (CLIA)		
	N	Positive	%Sensitivity
Systemic Lupus Erythematosus	87	52	59.8
Mixed Connective Tissue Disease	48	46	95.8
Sjögren's Syndrome	25	17	68.0
Systemic Sclerosis	18	10	55.6
Polymyositis/Dermatomyositis	11	3	27.3
Total	189	128	67.7

Clinical Specificity

The clinical specificity was determined for 308 specimens, consisting of 143 patients with other diseases (Primary Biliary Cirrhosis, Celiac disease, Rheumatoid Arthritis, Autoimmune Thyroiditis, Epstein-Barr Virus) and 165 apparently healthy individuals. The clinical specificity was calculated to be 98.1%. The following results were obtained:

Specimen Category	ENA Screen (CLIA)		
	N	Negative	%Specificity
Primary Biliary Cirrhosis	45	44	97.8
Celiac disease	13	13	100
Rheumatoid Arthritis	57	55	96.5
Autoimmune Thyroiditis	19	19	100
Epstein-Barr Virus	9	8	88.9
Apparently Healthy	165	163	98.8
Total	308	302	98.1

High-Dose Hook

No high-dose hook effect was seen for anti-ENA antibodies concentrations up to 4000 AU/mL.

Method Comparison

A comparison of the ENA Screen assay with a commercially available immunoassay, gave the following correlations (AU/mL):

Number of samples measured: 200

Passing-Bablok: y=1.0108x-0.3286, r=0.937.

The clinical specimen concentrations were between 4.171 and 159.764 AU/mL.

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
	Temperature limit (Store at 2-8°C)		Use-by date
	Contains sufficient for<n> tests		Keep away from sunlight
	This way up		Authorized representative in the European Community
	<i>In vitro</i> diagnostic medical device		Kit component
	Catalogue number		Batch code
	CE marking with notified body ID number		Reconstitute with

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