Human Sm antibody ELISA Kit

Enzyme Immunoassay for the quantification of IgG antibodies to Sm in serum or plasma.

Catalog number: ARG80417

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION   **Page**

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

Email: info@arigobio.com

Human Sm antibody ELISA Kit ARG80417

INTRODUCTION

Connective tissue diseases (CTD) are a group of autoimmune disorders which are characterized by presence of antinuclear antibodies (ANA) in the blood of patients. ANA are a specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the cells. These antibodies are involved in the disease pathogenesis, and they also constitute the basis for diagnosis and treatment of CTD.

ANA have been categorized into two main groups:

1. Autoantibodies to DNA and histones

2. Autoantibodies to extractable nuclear antigens (ENA): Sm, ribonucleoproteins (RNP), SSA/Ro, SSB/La, Scl-70, Jo-1 and PM1 Autoantibodies to DNA and histones include antibodies against single and double stranded DNA (ssDNA and dsDNA). Significant levels of anti-dsDNA antibodies are considered to be confirmatory in the diagnosis of systemic lupus erythematosus (SLE). Anti-histone antibodies are indicative of drug-induced lupus.

Besides DNA and histones, autoantibodies may also target other nuclear antigens. These nuclear antigens were named extractable nuclear antigens (ENA), as originally they were extracted from the nuclei with saline solution. Autoantibodies to Smith antigen (Sm) which is also considered to be highly specific for SLE were the first anti-ENA detected. Thereafter, further subtypes of ENA i.e. ribonucleoproteins (RNP), Sjögren antigen A or B (SSA/Ro or SSB/La), Scl-70, Jo-1 and PM1 were identified. Although most of these ENA are disease

specific, a significant overlap exists. Sensitivity and specificity may also vary depending upon the type of underlying CTD.Presence of autoantibodies in the sera of patients constitutes one of the criteria used for diagnosis of CTD. Together with the clinical diagnosis ANA subtyping helps in identifying a specific CTD.

Indirect immunofluorescence tests (IF) and enzyme immunoassays (ELISA) are commonly used for ANA detection in day to day practice. Initially, screening is carried out by IF-ANA or a generic ELISA which detects ANA of a broad specificity similar to IF-ANA. If positive, more specific tests are performed based on clinical findings and the IF-ANA staining pattern.

These antigen specific ELISA assays react with single autoantigens e.g. dsDNA, SS-A/Ro, SS-B/La, Scl-70, Sm, Sm/RNP etc. Autoantibodies to dsDNA are specific and diagnostic for SLE and levels are elevated during active disease. Recently published ACR Guidelines for Screening, Treatment, and Management of Lupus Nephritis recommend the testing of antibodies to dsDNA for monitoring of lupus nephritis, ranging from monthly intervals in pregnant patients with active glomerulonephritis at onset of treatment to every three months in patients with active nephritis at onset of treatment or pregnant

patients with previous but not current nephritis, up to six-monthly testing in patients with previous active nephritis or no prior or current nephritis. SLE-Patients without antibodies against dsDNA often produce antibodies against ssDNA. Similarly anti-Sm is highly specific for SLE but is present in only 10% to

30 % of SLE cases.

Antibodies against dsDNA, histones, the 70 kD protein of the U1-snRNP complex (RNP70) and anti Sm are closely associated with SLE. Anti-SSA/Ro and anti-SSB/La antibodies are indicative for Sjögren's syndrome, but can also be found in up to 30 % cases of SLE with cutaneous involvement. Anti-SS-A/Ro antibodies pass the placenta and may cause the development of SLE in neonates. Anti-SSA/Ro antibodies are almost always present in sera of mothers with babies with neonatal lupus syndrome and with complete congenital heart block.

Antinucleolar antibodies are a group of autoantibodies which give a nucleolar IF-staining pattern. Most common of these are anti-PM-Scl, anti-RNA polymerase I-III and anti-U3-RNP They are found in scleroderma and polymyositis (PM). Antibodies against RNP and the complex RNP/Sm are linked to mixed connective tissue disease (MCTD, Sharp syndrome) and to SLE. Serologically MCTD is characterized by the presence of autoantibodies directed against the 70 kD protein of the U1-snRNPcomplex. Up to 100% of MCTD patients manifest high titers of Anti-RNP-70 antibodies.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. Highly purified Sm has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated

human antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard	6 vials (0, 12.5, 25, 50, 100 , 200 U/ml)(Ready-to-use)	4°C
Controls	2 vials of Positive and Negative Controls (1.5 ml each) (Ready-to-use)	4°C
5X Sample buffer	20ml	4°C
HRP-Antibody conjugate (IgG)	15ml (Ready-to-use)	4°C
50X Wash buffer	20ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 50X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

<u>Serum</u>- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and

assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

<u>Plasma</u> - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.
- **1X Sample buffer:** Dilute 5X Sample buffer with distilled water before use.
- **Patient sample**: Dilute patient sample 1:100 with 1X sample buffer before assay, mix well. (e.g. 10 µl of sample + 990 µl of 1X sample buffer)

  Note: the controls and calibrators are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100µl of standards, controls, samples and zero controls into wells.
3. Incubate for 30 minutes at RT.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of

liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add 100µl 1X HRP-antibody conjugate into each well. Incubate for 15 minutes at RT.

6. Wash as according to step 4.

7. Add 100µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature.

8. Add 100µl of Stop Solution to each well. Incubate for 5 minutes at RT. The color of the solution should change from blue to yellow.

9. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

INTERPRETATION OF RESULTS

Negative: <15U/ml, Borderline: 15-25U/ml, Positive: >25U/ml

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Sm Ab ranged from 12.5-200 U/ml.
The mean MDD was 1 U/ml.

Interference

No interference has been observed with the following factors:

Haemolytic sera/plasma (up to 1000mg/dl)

Lipemic sera/plasma (up to 3g/dl triglycerides)

Bilirubin containing sera/plasma (up to 40mg/dl)

Antiboagulants (Citrate, EDTA, Heparin).

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.9% and inter-assay precision was 3%.