Human RNP 70 antibody ELISA Kit

Enzyme Immunoassay for the quantification of autoantibodies to RNP 70 in serum or plasma.

Catalog number: ARG80414

Package: 96 wells

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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, ribonucleo-proteins (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.

Antinuclear 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-snRNP-complex. 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 recombinant RNP 70 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 0-5 (0, 12.5, 25, 50, 100 , 200 U/ml)	6 X 1.5 ml (Ready-to-use)	4°C
Control 1(Positive Control; Accept range: 40-70 U/ml)	1.5 ml (Ready-to-use)	4°C
Control 2 (Negative Control; Accept range: < 15 U/ml)	1.5 ml (Ready-to-use)	4°C
5X Sample buffer	20 ml	4°C
HRP-Antibody conjugate (IgG)	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: reference filter at 620 nm)
- 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 in the dark at all times. Do not expose reagents to heat, sun, or strong light during storage and usage.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- All materials must be at room temperature (20-28°C) prior to use.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°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.
- All incubation steps must be accurately timed.
- 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.

Serum- 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 for up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using Citrate EDTA or heparin 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 for up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
b) Samples containing sodium azide should not be used in the assay.
c) Testing of heat-inactivated sera is not recommended.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 20 ml of 50X Wash buffer + 980 ml of distilled water) The diluted Wash buffer is stable for 30 days at 2°C to 8°C.
- 1X Sample buffer: Dilute 5X Sample buffer with distilled water before use. (E.g. 20 ml of 5X Sample buffer + 80 ml of distilled water) The diluted Sample buffer is stable for 30 days at 2°C to 8°C.
- 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, 20-28 °C) 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 **100 µl** of **standards, controls and prediluted samples** into wells.
3. Incubate for **30 minutes at RT (20-28 °C)**.
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300µl)** 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 **100 µl** of **HRP-Antibody conjugate** solution into each well. Incubate for **15 minutes at RT**.
6. **Wash** as according to step 4.
7. Add **100 µl** of **TMB Reagent** to each well. Incubate for **15 minutes at room temperature in dark**.
8. Add **100 µl** 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 **450 nm** immediately (optional: Read the OD at 600-690nm as reference). It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. 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.

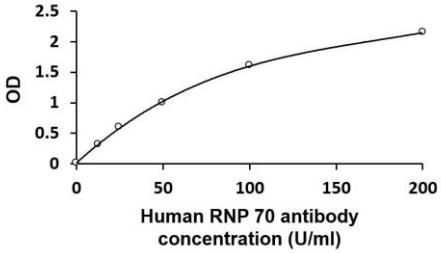
5. If these quality control criteria are not met the assay run is invalid and should be repeated.

INTERPRETATION OF RESULTS

Negative: < 25 U/ml; Positive: ≥ 25 U/ml

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of RNP 70 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 5.1% and inter-assay precision was 5.1%.