RNP 70 Ab ELISA Kit

Catalog Number KA1278

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

RNP 70 Ab ELISA Kit is an enzyme immunoassay for quantitative determination of IgG Auto-antibodies to RNP 70 in human serum or plasma.

Background

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-snRNP-complex. Up to 100% of MCTD patients manifest high titers of Anti-RNP-70 antibodies.

Autoantibody prevalence to (values in %)

Diseases	dsDNA	ssDNA	Histone	SS-A	SS-B	Sm	RNP/Sm	Scl-70	Jo-1
Systemic lupus erythrematosus (SLE)	> 90	> 90	30-50	10-30	30-50	10-30	10-30		
Drug-induced lupus (DIL)		30-50	50-90						
Sharp-syndrome/ mixed connective tissue disease	10-30	10-30					> 90		
Rheumatoid arthritis	10-30	30-50	30-50	10-30					
Sjögren´s syndrome	10-30	10-30		> 90	> 90				
Scleroderma	10-30	10-30		10-30				> 90	
Photosensitive dermatitis, dermatomyositis	10-30	10-30							50-90

Principle of the Assay

Recombinant RNP 70 is bound to microwells. Antibodies against the coated antigen, if present in diluted sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

General Information

Materials Supplied

List of component

Component	Amount
Divisible microplate consisting of 12 modules of 8 wells each. Ready to use.	96 (8x12) wells
Calibrators A-F (0, 12.5, 25, 50, 100, 200 U/mL) containing serum/buffer matrix (PBS, BSA detergent, NaN_3 0.09%), yellow. Ready to use.	1.5 mL x 6
Control positive (1) and negative (2), containing RNP-70 antibodies in serum/buffer matrix (PBS, BSA detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.	1.5 mL x 2
Sample Buffer P, containing PBS, BSA detergent, preservative NaN_3 0.09%, yellow. 5x concentrate.	20 mL
Enzyme conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA detergent, preservative ProClin 300 0.05%, light red. Ready to use.	15 mL
TMB substrate containing 3,3',5,5'-Tetramethylbenzidin, colorless. Ready to use	15 mL
Stop solution (contains acid). Ready to use	15 mL
Wash solution containing Tris, detergent, preservative NaN_3 0.09%. 50x concentrate.	20 mL

Storage Instruction

- ✓ Store the kit at 2-8°C in the dark.
- ✓ Do not expose reagents to heat, sun or strong light during storage and usage.
- ✓ Store microplate sealed and desiccated in the clip bag provided.
- ✓ Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- ✓ Diluted Wash Solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Materials Required but Not Supplied

- ✓ Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- ✓ Data reduction software
- ✓ Multi-Channel Dispenser or repeatable pipet for 100 µL
- ✓ Vortex mixer
- ✓ Pipets for 10 µL, 100 µL and 1000 µL
- ✓ Laboratory timing device
- ✓ Distilled or deionized water
- ✓ Measuring cylinder for 1000 mL and 100 mL

- ✓ Plastic container for storage of the wash solution
- ✓ The ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

Precautions for Use

- Procedural Notes

- ✓ Do not use kit components beyond their expiration dates.
- ✓ Do not interchange kit components from different lots and products.
- ✓ All materials must be at room temperature (20-28°C) prior to use.
- ✓ Prepare all reagents and samples. Once started, perform the test without interruption.
- ✓ Double determinations may be done. By this means pipetting errors may become obvious.
- ✓ Perform the assay steps only in the order indicated.
- ✓ Always use fresh sample dilutions.
- ✓ Pipette all reagents and samples into the bottom of the wells.
- ✓ To avoid carryover or contaminations, change the pipette tip between samples and different kit controls.
- ✓ Wash microwells thoroughly and remove the last droplets of Wash Solution.
- ✓ All incubation steps must be accurately timed.
- ✓ Do not re-use microplate wells.

- Warnings and Precautions

- ✓ All reagents of this kit are intended for professional use only.
- ✓ Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2 and so all human serum based reagents in this kit must be handled as through capable of transmitting infection.
- ✓ Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- ✓ Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
- ✓ Stop Solution contains acid, classification is non hazardous. Avoid contact with skin.
- ✓ Calibrators, Controls, Sample Buffer and Wash Solution contain sodium azide (NaN_3) 0.09% as preservative. This concentration is classified as non-hazardous.
- ✓ Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
- ✓ During handling of all reagents, controls, and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice.
- ✓ First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- ✓ Personal precautions, protective equipment and emergency procedures.

- ✓ Observe laboratory safety regulations. Avoid contact with skin, and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- ✓ Exposure controls/personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions know.
- ✓ Conditions to avoid: Since substrate solution is light-sensitive. Store in dark.
- ✓ For disposal of laboratory waste the national or regional legislation has to be observed.
- ✓ Observed the guidelines for performing quality control in laboratory by assaying control sera.

Assay Protocol

Reagent Preparation

✓ Wash Solution

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 mL prior to use.

✓ Sample Buffer

Sample Buffer P: Prior to use dilute the contents (20 mL) of one vial of the sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 mL.

Sample Preparation

✓ Specimens collection, storage and handling

1. Collect whole blood specimens using acceptable techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum or plasma by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
6. Testing of heat-inactivated sera is not recommended.

✓ Preparation of samples

Dilute samples 1:100 before assay. Put 990 µL of prediluted sample buffer in a polystyrene tube and add 10 µL of sample. Mix well. *Note: Calibrators/Controls are ready to use and need not be diluted.*

Assay Procedure

Assay Procedure

Prepare enough microplate modules for all calibrators / controls and samples.

1. Pipette 100 µL of calibrators, controls and prediluted samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28°C).
3. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
4. Dispense 100 µL of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.

6. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
7. Dispense 100 µL of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature.
9. Add 100 µL of stop solution to each well of the modules.
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Data Analysis

Calculation of Results

- ✓ Validation

  Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each kit.
  If these quality control criteria are not met the assay run is invalid and should be repeated.

- ✓ Calculation of results

  For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of samples may then be estimated from the calibration curve by interpolation.
  Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Performance Characteristics

- ✓ Calibration

  This assay system is calibrated in relative arbitrary units, science no international reference preparation is available for this assay.

- ✓ Measuring Range

  The calculation range of this ELISA assay is 0-200 U/mL.

- ✓ Expected values:

  In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 25 U/mL.

- ✓ Interpretation of results

Negative	< 25 U/mL
Positive	≥ 25 U/mL

✓ Linearity

Samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed Index (U/mL)	Expected Index (U/mL)	O/E (%)
1	1:100	161.4	161.4	100
-	1:200	78.0	80.7	97
-	1:400	39.7	40.4	98
-	1:800	20.1	20.2	100
2	1:100	167.2	167.2	100
-	1:200	83.7	83.6	100
-	1:400	41.5	41.8	99
-	1:800	20.8	20.9	100

✓ Limit of detection

Functional sensitivity was determined to be: 1 U/mL

✓ Interfering Substances

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) sera or plasma, or bilirubin (up to 40 mg/dL) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

✓ Reproducibility

- Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
- Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay			Inter-Assay		
Sample	Mean U/mL	CV%	Sample	Mean U/mL	CV%
1	12.8	5.8	1	14.4	4.9
2	105.0	3.9	2	108.7	5.2
3	182.0	5.7	3	175.2	5.2

✓ Study Results

Study Population	n	n Pos	%
SLE	70	37	52.9
MCTD	30	29	96.7
Rheumatoid Arthritis	20	3	15.0
Normal human sera	100	2	2.0

IgG	Pos	Neg	
Pos	66	5	
Neg	34	115	
	100	120	220

Sensitivity: 66.0%

Specificity: 95.8%

Overall agreement: 82.3%

Resources

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Calibrator A	Sample										
B	Calibrator B	Sample										
C	Calibrator C	Sample										
D	Calibrator D	Sample										
E	Calibrator E	Sample										
F	Calibrator F	Sample										
G	Control Positive	Sample										
H	Control Negative	Sample										