Version 06-06/24

User's Manual

Human RNP/Sm IgG ELISA

REF **DEIA05728-1**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Human RNP/Sm IgG is an indirect ELISA for the detection and quantitation of IgG class of antibodies against RNP/Sm antigen in human serum or plasma. This kit is for in vitro research use only (RUO), and not for therapeutic use.

General Description

Rheumatoid autoimmune diseases are often associated with the occurrence of autoantibodies against several nuclear or cytoplasmatic antigens. These so-called anti nuclear antigens (ANA) can be divided into three groups:

1. true anti nuclear antigens (ANA): dsDNA, ssDNA, histones, nucleolic RNA and DNP
2. extractable nuclears antigens: Sm (Smith), n-RNP, Scl 70 and PM-1
3. cytoplasmatic antigens: SS-A (Ro)*, SS-B (La)* and Jo-1 SS-A (Ro) and SS-B (La) are co-localized in cytoplasm and nucleus

Inflammatory connective tissue diseases are characterized by idiopathic genesis along with disturbances in terms of cellular and humoral immunity, systemic organ failure and a chronic course of disease. Additionally, connective tissue diseases exhibit overlapping symptomatic features that render an accurate diagnosis difficult. Considering the diversity of mixed connective tissue diseases, such disorders exhibit a common serological characteristic; the presence of anti-nuclear antibodies. These antibodies are directed against parts of the cell nucleus and the cytoplasm, and many rheumatic diseases are characterized by the presence of one or more of these ANAs. Antibodies to doublestranded DNA (dsDNA), single-stranded DNA (ssDNA), histone, nuclear ribonucleoprotein (RNP) and Smith antigen (Sm) are associated with SLE, while antibodies to Sjogren´s Syndrome A (SSA/Ro) and Sjogren´s Syndrome B (SSB/La) can occur in both SLE and Sjogren´s Syndrome (SS). Antibodies to Jo-1 may be observed in polymyositis and dermatomyositis, while antibodies to scleroderma- associated antigen (Scl-70) and centromere can occur in patients with progressive systemic sclerosis (PSS). Anti-histone antibodies are associated with SLE and druginduced lupus, while anti-RNP antibodies are linked with mixed connective tissue disease (MCTD) and with SLE. Antibodies directed against centromere are associated with CREST syndrome. Although IFA technology was traditionally used to detect autoantibodies in conjunction with HEp2 cells, it is now widely acknowledged that ELISA technology offers an excellent alternative.

Anti-Nuclear Antibodies (ANA) are autoantibodies which binds to cellular nuclear antigens including ds-DNA, ss-DNA, histones, ribonucleoproteins (RNP) and the SS-A, SS-B, and Sm antigens. ANA ELISA, a sandwich ELISA, provides a rapid semi-quantitative measurement of ANA in serum to further investigate the presence of specific autoantibodies

Principles of Testing

RNP/Sm IgG ELISA kit is based on binding of RNP/Sm IgG from serum samples to human gamma globulin immobilized on microtiter wells. After a washing step, anti-human IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of RNP/Sm IgG present in

the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of RNP/Sm IgG in samples is calculated on the basis of the absorbance of the negative, positive, and, calibrator controls.

Reagents And Materials Provided

Purified RNP/Sm coated microwell strips (96 wells), Readyto-use, 1 Plate

Anti-RNP/Sm Standard A (0 U/ml) 1.5 ml, 1 vial

Anti-RNP/Sm Standard B (12.5 U/ml) 1.5 ml, 1 vial

Anti-RNP/Sm Standard C (25 U/ml) 1.5 ml, 1 vial

Anti-RNP/Sm Standard D (50 U/ml) 1.5ml, 1 vial

Anti-RNP/Sm Standard E (100 U/ml) 1.5 ml, 1 vial

Anti-RNP/Sm Standard F (200 U/ml) 1.5 ml, 1 vial

Anti-RNP/Sm Postive control, 1.5 ml, 1 vial

Anti-RNP/Sm Negative control, 1.5 ml, 1 vial

Anti-RNP/Sm Sample Buffer (5×), 20 ml, 1 bottle

Anti-hIgG HRP Conjugate, 15 ml, 1 bottle

Wash buffer (50×), 20 ml, dilute 1:50 with distilled water, 1 bottle

HRP Substrate Solution, 15 ml, 1 bottle

Stop solution, 15 ml,1 bottle

Complete Instruction Manual; 1

Materials Required But Not Supplied

Adjustable micropipet (20-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

Storage

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions.

Specimen Collection And Preparation

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed , these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Reagent Preparation

Wash buffer is supplied as 50× stock. Dilute 20 ml into 980 ml deionized or distilled water, mix, and store at room temp for 1-2 weeks. It can be stored at 4°C for long term storage.

Sample Buffer (5×): Dilute 20 ml into 80 ml deionized or distilled water.

Dilute serum sample 1:100 in 1× sample diluent (5 µl sample in 495 µl buffer) .

Assay Procedure

Label or mark the microtiter well strips to be used on the plate. Dilute controls, calibrators, and serum samples 1:100 (5 µl of sample in a total volume of 500 µl of sample diluents). Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter). Dilute Sample Diluent (5×): Dilute 20 ml into 80 ml de-ionized or distilled water. Standards and controls are supplied pre-diluted.

1. Pipet 100 µl of diluted sample diluents, negative & positive controls, calibrator, and diluted serum samples into appropriate wells in duplicate. Cover the plate and incubate for 30 minutes at room temperature (20-28°C).
2. Aspirate and wash the wells 3 times with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
3. Add 100 µl of antibody-enzyme conjugate into each well. Mix gently. Cover the plate and incubate for 15 minutes at room temperature(20-28°C).
4. Aspirate and wash the wells 3 times with 300 µl of diluted wash buffer, as above.
5. Dispense 100 µl TMB substrate per well. Mix the plate gently for 5-10 seconds. Cover the plate and incubate for 15 minutes at room temperature. Blue color develops into standards and positive samples.
6. Stop the reaction by adding 100 µl of stopping solution to all wells at the same timed intervals as in step 8. Mix gently. Blue color turns yellow.
7. Measure the absorbance at 450 nm using an ELISA reader.

Note: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

Calculation

For the RNP/Sm test a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is recommended. Spline Approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Interpretation Of Results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-Sm test:

Anti-Sm/RNP [U/ml]

normal: < 15

borderline: 15 - 25

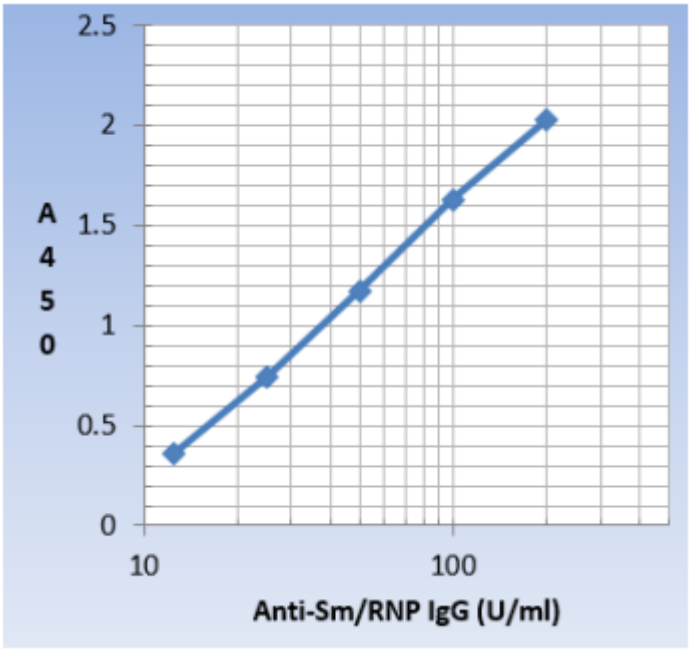
elevated: > 25

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-Sm antibodies. The above reference ranges should be regarded as guidelines only.

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA, since no other international standards are available.

Typical Standard Curve

These data are for demonstration purpose only.



Precision

Intra-assay precision:

Sample	Mean (U/ml)	CV%
1	35.6	4.1
2	101.9	5.9
3	182.0	1.8

Inter-assay precision:

Sample	Mean (U/ml)	CV%
1	33.3	4.2
2	109.6	3.1
3	176.8	2.9

Sensitivity

The lower detection limit for the Anti-RNP/Sm test was determined at 1 U/ml.

Specificity

The microplate is coated with RNP/Sm highly purified by affinity chromatography. The Anti RNP/Sm test kit is specific only for autoantibodies directed to RNP/Sm. No crossreactivities to the other ENA-antigens have been observed.

Species Crossrectivity

This kit is recommended for human samples only. Its utility in other species such as mouse, rat, or monkey etc has not been tested. CD has a separate Sm/RNP ELISA kit for mouse, rat, and monkey samples.