ELISA kits available from ADI (see details at the web site)

3100	Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105	Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3115	Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205	Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-
3210-SSA	Human anti-SS-A/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB	Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3250	Human Anti-thryroid peroxidase ELISA kit, Semi-Quantitative
3300	Human Anti-helicobacter pylori IgG ELISA kit, Semi-Quantitative
3300-100-SMG	Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG	Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG	Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG	Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG	Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-150-JOG	Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-160-AFG	Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG	Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G	Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M	Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-APS	Human Anti-Phospholipid Screen IgG/IgM ELISA kit, 96 tests,
3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests,
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests,
3300-220-PAS	Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests,
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-270-GBG	Human Anti-glomerular basement membrane (GBM) IgG ELISA kit, 96
3300-280-BPG	Human Anti-bactericidal permeability increasing (BPI) protein IgG
3300-290-ELG	Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
3300-300-GLG	Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
3300-305-GLM	Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
3300-310-GLA	Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
3300-315-PRG	Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H//K/ATPase) IgG ELISA kit, 96 tests,
3300-320-ASC	Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG
3300-330-ASG	Human Anti-Sperm IgG ELISA kit, 96 tests, Quantitative
3300-340-CCG	Human Anti-Cyclic Citrullinated Peptide (CCP)IgG ELISA kit, 96 tests,
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit, 96 tests,
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests, Quantitative
3310	Human Anti-helicobacter pylori IgM ELISA kit, Semi-Quantitative
3320	Human Anti-helicobacter pylori IgA ELISA kit, Semi-Quantitative
3600-HIG	Human Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3610-MKG	Monkey Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3700-MIG	Mouse Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3710-MIM	Mouse Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
3750-RIG	Rat Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3760-RIM	Rat Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
4000	Mouse Anti-Myelin Oligodendrcyte protein (MOG35-55) Ig's ELISA kit,

Human Anti-RNP (RNP-70) IgG Elisa Kit

ELISA KIT Cat. # 3300-120-RNG

For Quantitative Determination of human IgG class antibodies against RNP-70 in serum or plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Human Anti-RNP (RNP-70) IgG # 3300-120-RNG

For Quantitative Determination of anti-RNP (RNP-70).

Kit Contents: (reagents for 96 tests)

Components	
Recombinant RNP-70 coated microwell strips (96 wells), Ready-to-use, 3300121-P	1 Plate
Anti-RNP-70 **Standard A** (0 U/ml) 1.5 ml, #3300122A	1 vial
Anti- RNP-70 **Standard B** (12.5 U/ml) 1.5 ml, #3300122B	1 vial
Anti-RNP-70 **Standard C** (25 U/ml) 1.5 ml, #3300122C	1 vial
Anti-RNP-70 **Standard D** (50 U/ml) 1.5 ml, #3300122D	1 vial
Anti-RNP-70 **Standard E** (100 U/ml) 1.5 ml, #3300122E	1 vial
Anti-RNP-70 **Standard F** (200 U/ml) 1.5 ml, #3300122F	1 vial
Anti-RNP-70 **Postive control**, 1.5 ml, #3300122PC	1 vial
Anti-RNP-70 **Negative control**, 1.5 ml, #3300122NC	1 vial
Anti-RNP-70 **Sample Diluent** (5X), 20 ml, #3300123	1 bottle
Anti-hIgG **HRP Conjugate**, 15 ml, #3300124	1 bottle
Wash buffer (50X), 20 ml, # 3300120-WB dilute 1:50 with distilled water,	1 bottle
HRP Substrate Solution, 15 ml, #3300120-TM	1 bottle
Stop solution, 15 ml, # 3300120-ST	1 bottle
Complete Instruction Manual; M-3300-120-RNG	1

Intended Use

ADI's Human Anti-Thyroglobulin (RNP-70) IgG ELISA kit is an indirect solid phase enzyme immunoassay (ELISA) for the determination of IgG class autoantibodies against RNP-70 in human serum or plasma. For in vitro research use only (RUO).

Introduction

Autoantibodies against RNP-70, the 70 kDa protein of the U1-snRNP complex, are known markers for the serological detection of Sharp syndrome (mixed connective tissue disease, MCTD). The diagnostic sensitivity of the marker is 100 %; the absence of RNP-70 autoantibodies completely rules out MCTD. For systemic lupus erythematosus (SLE), antibodies against RNP-70 are detectable in up to 32 % of cases; in systemic scleroderma they are found in up to 10 % of cases.

Patients whose only detectable antinuclear antibodies (ANA) are autoantibodies against the RNP-70 protein have the classical presentation of MCTD. If additional SLE-typical autoantibodies (anti-dsDNA, anti-Sm, anti-histone) are found, transition into SLE is indicated.

Precision (Reproducibility):

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations of each sample.

PERFORMANCE CHARACTERISTICS

Intra-assay precision:

Sample	Mean (U/ml)	**CV%**
1	12.8	5.8
2	105.0	3.9
3	182.0	5.7

Inter-assay precision:

Sample	Mean (U/ml)	**CV%**
1	14.4	4.9
2	108.7	5.5
3	175.2	5.2

Sensitivity:

The lower detection limit for the Anti-RNP-70 test was determined at 1 U/ml.

Specificity:

The microplate is coated with recombinant RNP-70 as antigen. The Anti-RNP-70 test kit recognizes only autoantibodies specific to the concerning antigen. No cross reactivities to other autoantigens and autoantigens have been observed.

Calibration:

Since no international reference preparation for Anti-RNP-70 autoantibodies is available, the assay system is calibrated in relative arbitrary units.

LIMITATIONS OF PROCEDURE

The Anti-RNP-70 ELISA is intended for research use only – not for use in diagnostic procedures. A negative Anti-RNP-70 result does not rule out the presence of SLE or MCTD.

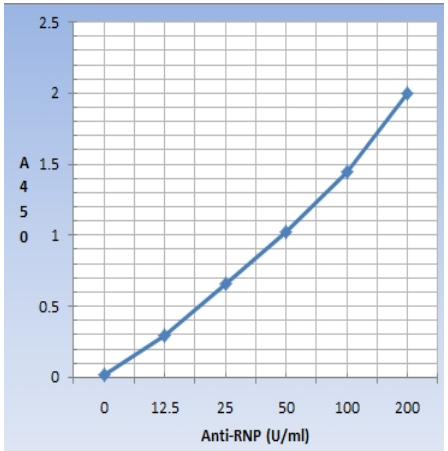
INTERFERING SUBSTANCES

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

WORKSHEET OF TYPICAL ASSAY

Wells	Stds (U/ml)	Mean $A_{450\ nm}$	Calcul. Conc. (U/ml)*
A1, A2	0	0.016	
B1, B2	12.5	0.298	
C1, C2	25	0.562	
D1, D2	50	0.893	
E1, E2	100	1.549	
F1, F2	200	2.002	

NOTE: These data are for demonstration purpose only. A complete set of negative, positive, and calibrator standards set must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



2-ADI_ELISA

CALCULATION OF RESULTS

Quality Control:

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not fulfil-led, the results are invalid and the test should be repeated.

For Anti-RNP-70 IgG ELISA a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

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.

PRINCIPLE OF THE TEST

Anti-RNP (RNP-70) ELISA kit is based on binding of RNP-70 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-70 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-70 in samples is calculated on the basis of the absorbance of the negative, positive, and, calibrator controls.

MATERIALS AND EQUIPMENT REQUIRED

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.

PRECAUTIONS

The Alpha Diagnostic International Anti-RNP (RNP-70) IgG ELISA Kit is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Negative, Positive, and Calibrator controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

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.

REAGENTS PREPARATIONS:

Wash buffer is supplied as **50x stock**. Dilute **20 ml into 980** ml de-ionized or distilled water, mix, and store at room temp for 1-2 weeks. It can be stored at 4oC for long term storage.
Sample Diluent (5X): **Dilute 20 ml into 80 ml de-ionized or distilled water.**

Dilute serum sample 1:100 in 1x sample diluent (5 ul sample in 495 ul buffer) .

STORAGE AND STABILITY

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.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

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 (5X)**: **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** (20-28oC).

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(20-28oC).

4. Aspirate and wash the wells **3 times** with 300 µl of diluted wash buffer, as above.

5. Dispense **100 ul 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.

8. 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.

9. Measure the absorbance at 450 nm using an ELISA reader.

NOTES

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.