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

**Human RNP/Sm IgG Elisa Kit**

**ELISA KIT # 3300-110-SRG**

For Quantitative Determination of RNP/Sm Antibodies in human serum or plasma

For In Vitro Research Use Only

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**Product Information**

**Catalog#** ProdDescription
2940-10 Human C1q ELISA Kit, 96 tests
2950 Human Anti-C1q IgG ELISA Kit, 96 tests
2960 Human Circulating Immune complexes (CIC) ELISA Kit, 96 tests
2970 Monkey Circulating Immune complexes (CIC) ELISA Kit, 96 tests
3000 Human Rheumatoid Factors IgM (RF) ELISA Kit, 96 tests, Semi-Quantitative
3100 Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105 Human anti-dsDNA IgM 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-Quantitative
3210-SA Human anti-SS-A (60 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3215-SA Human anti-SS-A (52 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3310 Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3300-100-SMG Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
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 ELISA
3300-150-JOG Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit,
3300-160-AFG Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG Human Anti-Cardioliopin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM Human Anti-Cardioliopin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA Human Anti-Cardioliopin IgA ELISA kit, 96 tests, Quantitative
3300-190-BZG Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-BZM Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-BZA Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-205-APS Human Anti-Phospholipid Screen (anti-Phosphatidyl Serine, Phosphatidyl
Inositol, Phosphaticid Acid and beta-2-Glycoprotein I) IgG/IgM ELISA kit, 96 tests, Quantitative
3300-210-PSS Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests, Quantitative
3300-215-PIS Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests, Quantitative
3300-220-PAS Human Anti-Phosphotidyl Acid IgG/IgM ELISA kit, 96 tests, Quantitative
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 tests,
3300-255-PRG Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-260-LPG Human Anti-Lactoferrin IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG Human ANCA (Anti-MPO) IgG 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, Quantitative
5120 Mouse anti-dsDNA IgG-specific ELISA Kit, 96 tests, Quantitative
5130 Mouse anti-dsDNA IgM-specific ELISA Kit, 96 tests, Quantitative
5210 Mouse Anti-Nuclear Antigens (ANA/ENA) Ig's (total (A+G+M) ) ELISA Kit, 96 tests,
5320 Mouse Anti-ssDNA IgG-specific ELISA Kit, 96 tests, Quantitative
5330 Mouse Anti-ssDNA IgM-specific ELISA Kit, 96 tests, Quantitative
5405 Mouse Anti-Sm Ig's (total (A+G+M) ELISA Kit, 96 tests, Quantitative
5415 Mouse Anti-rRNP IgG ELISA Kit, 96 tests, Quantitative
5420 Mouse Anti-rRNP IgM ELISA Kit, 96 tests, Quantitative
5520 Rat Anti-Cardioliopin Ig's (A+G+M) ELISA Kit, 96 Tests, Quantitative
5610 Mouse Anti-Histones Ig's (total (A+G+M) ELISA Kit, 96 tests, Quantitative
5710 Mouse Anti-SSA/Ro Ig's (total (A+G+M) ELISA Kit, 96 tests, Quantitative
5810 Mouse Anti-SSB Ig's (total (A+G+M) ELISA Kit, 96 tests, Quantitative
5900 Mouse Circulating Immune Complexes (CIC) Ig's (total (A+G+M) ELISA kit, 96 Tests,
5950 Rat Circulating Immune Complexes (CIC) Ig's (total (A+G+M) ELISA kit, 96 Tests,
Human RNP/Sm IgG # 3300-110-SRG

Kit Contents: (reagents for 96 tests)

<table>
<thead>
<tr>
<th>Components</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified RNP/Sm coated microwell strips</td>
<td>96 wells, Ready-to-use, 3300-111P</td>
</tr>
<tr>
<td>Anti-RNP/Sm Standard A (0 U/ml)</td>
<td>1 ml, #3300-102A</td>
</tr>
<tr>
<td>Anti-RNP/Sm Standard B (12.5 U/ml)</td>
<td>1 ml, #3300-112B</td>
</tr>
<tr>
<td>Anti-RNP/Sm Standard C (25 U/ml)</td>
<td>1 ml, #3300-112C</td>
</tr>
<tr>
<td>Anti-RNP/Sm Standard D (50 U/ml)</td>
<td>1 ml, #3300-112D</td>
</tr>
<tr>
<td>Anti-RNP/Sm Standard E (100 U/ml)</td>
<td>1 ml, #3300-112E</td>
</tr>
<tr>
<td>Anti-RNP/Sm Standard F (200 U/ml)</td>
<td>1 ml, #3300-112F</td>
</tr>
<tr>
<td>Anti-RNP/Sm Positive control</td>
<td>1.5 ml, #3300-112PC</td>
</tr>
<tr>
<td>Anti-RNP/Sm Negative control</td>
<td>1.5 ml, #3300-112NC</td>
</tr>
<tr>
<td>Anti-RNP/Sm Sample Buffer (5X)</td>
<td>20 ml, #3300-113</td>
</tr>
<tr>
<td>Anti-IgG HRP Conjugate</td>
<td>15 ml, #3300-114</td>
</tr>
<tr>
<td>Wash buffer (50X)</td>
<td>20 ml, #3300110-WB</td>
</tr>
<tr>
<td>HRP Substrate Solution</td>
<td>15 ml, #3300110-TM</td>
</tr>
<tr>
<td>Stop solution, 15 ml</td>
<td>#3300110-SS</td>
</tr>
<tr>
<td>Complete Instruction Manual</td>
<td>M-3300-110-SRG</td>
</tr>
</tbody>
</table>

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.

Introduction

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

1. true anti nuclear antigens (ANA): dsDNA, ssDNA, histones, nucleolice RNA and DNP
2. extractable nuclear 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 double-stranded 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-

References:

2. Froelich, Ch. J., Wallmann, H., Skosey, J. L. and Teodorescu, M. Clinical Value of an Integrated ELISA System for the Detection of 6 Autoantibodies (ssDNA,dsDNA, Sm, RNP/Sm, SSA and SSB); The Journal of Rheumatology 1990; Vol 17, No 2: 192 – 200
WORKSHEET OF TYPICAL ASSAY

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds (U/ml)</th>
<th>Mean A_450 nm</th>
<th>Calcul. Conc. (U/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>0</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>B1, B2</td>
<td>12.5</td>
<td>0.361</td>
<td></td>
</tr>
<tr>
<td>C1, C2</td>
<td>25</td>
<td>0.744</td>
<td></td>
</tr>
<tr>
<td>D1, D2</td>
<td>50</td>
<td>1.174</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>100</td>
<td>1.769</td>
<td></td>
</tr>
<tr>
<td>F1, F2</td>
<td>200</td>
<td>2.024</td>
<td></td>
</tr>
</tbody>
</table>

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.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the sample diluents from the mean absorbance values of negative & positive controls, calibrator, and samples. 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.

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.

PRINCIPLE OF THE TEST

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.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µ) 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 Rheumatoid Factor IgM 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 4°C for long term storage.

**Sample Buffer (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 (20-28°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 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.

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.

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.

Calculation of results

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:

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<th></th>
</tr>
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<td>normal</td>
<td>&lt; 15</td>
</tr>
<tr>
<td>borderline</td>
<td>15 - 25</td>
</tr>
<tr>
<td>elevated</td>
<td>&gt; 25</td>
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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.

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