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

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-SSA	Human anti-SS-A (60 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3215-SSA	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
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, 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 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-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-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-205-APS	Human Anti-Phospholipid Screen (anti-Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic 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-Phosphotidic 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-LFG	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-nRNP IgG ELISA Kit, 96 tests, Quantitative
5420	Mouse Anti-nRNP IgM ELISA Kit, 96 tests, Quantitative
5520	Rat Anti-Cardiolipin 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,

Instruction Manual No. M-3300-100-SMG

Human Anti-Smith antigen (Sm) IgG Elisa Kit

ELISA KIT # 3300-100-SMG

For Quantitative Determination of anti-Sm Antibodies in human 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-Smith antigen (Sm) IgG # 3300-100-SMG

Kit Contents: (reagents for 96 tests)

Components	
Purified Sm coated microwell strips (96 wells), Ready-to-use, 3300-101P	1 Plate
Anti-Sm **Standard A** (0 U/ml) 1.5 ml, #3300-102A	1 vial
Anti-Sm **Standard B** (12.5 U/ml) 1.5 ml, #3300-102B	1 vial
Anti-Sm **Standard C** (25 U/ml) 1.5 ml, #3300-102C	1 vial
Anti-Sm **Standard D** (50 U/ml) 1.5 ml, #3300-102D	1 vial
Anti-Sm **Standard E** (100 U/ml) 1.5 ml, #3300-102E	1 vial
Anti-Sm **Standard F** (200 U/ml) 1.5 ml, #3300-102F	1 vial
Anti-Sm **Postive control**, 1.5 ml, #3300-102PC	1 vial
Anti-Sm **Negative control**, 1.5 ml, #3300-102NC	1 vial
Anti-Sm **Sample Diluent** (5X), 20 ml, #3300-103	1 bottle
Anti-hIgG **HRP Conjugate**, 15 ml, #3300-104	1 bottle
Wash buffer (50X), 20 ml, # 3300100-WB dilute 1:50 with distilled water,	1 bottle
HRP Substrate Solution, 15 ml, # 3300100-TM	1 bottle
Stop solution, 15 ml, # 3300100-SS	1 bottle
Complete Instruction Manual; M-3300-100-SMG	1

Intended Use

Human Anti-Smith antigen (Sm) IgG is an indirect ELISA for the detection and quantitation of IgG class of antibodies against Sm antignes 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 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 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-

PERFORMANCE CHARACTERISTICS

PRECISION

Intra-assay precision:

Sample	Mean (U/ml)	**CV%**
1	32.2	2.7
2	73.2	2.6
3	134.0	3.6

Inter-assay precision:

Sample	Mean (U/ml)	**CV%**
1	33.8	6.4
2	71.3	6.2
3	133.1	1.1

Sensitivity:

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

Parallelism:

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-Sm kit. The assay showed linearity over the full measuring range.

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. ADI has a separate ANA ELISA kit for mouse, rat, and monkey samples.

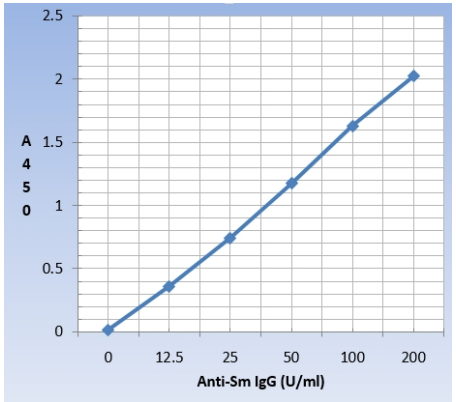
References:

1. Antinuclear Antibodies; The Lancet 1984; 15: 611 - 613.
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

Wells	Stds (U/ml)	Mean $A_{450\ nm}$	Calcul. Conc. (U/ml)*
A1, A2	0	0.017	
B1, B2	12.5	0.361	
C1, C2	25	0.744	
D1, D2	50	1.174	
E1, E2	100	1.769	
F1, F2	200	2.024	

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_Graphs

Recommended Lin-Log Plot

For quantitative results plot the optical density of each std versus the std concentration to create a calibration curve. The concentration of patient 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.

associated antigen (Scl-70) and centromere can occur in patients with progressive systemic sclerosis (PSS). Anti-histone antibodies are associated with SLE and drug-induced 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

PRINCIPLE OF THE TEST

Sm IgG ELISA kit is based on binding of 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 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 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 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 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 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.

Calculation of results

For the Anti-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 std 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[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.