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

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	**#0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

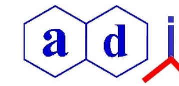
Instruction Manual No. M-3210-SSA

Anti- Sjögren syndrome type A antigen (SSA/Ro) IgG

ELISA KIT Cat. No. 3210-SSA

For Quantitative Determination of Anti-SSA IgG In Human Serum

For In Vitro Research Use Only



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

Anti SSA IgG ELISA KIT #3210-SSA

For Quant. Determination of Anti-SSA IgG In Human Serum: Kit Contents (96 tests):

Components	Cat. No.
Nuclear antigen coated microwell strips (96 wells)	3211
Anti-SSA IgG Stds. A-F in a serum buffer (0, 12.5, 25, 50, 100, and 200 U/ml) 6 vials x 1.5 ml each	3212A-F
Anti-SSA IgG Negative Control (1.5 ml each) Anti-SSA IgG Positive Control (1.5 ml each)	3213N 3213P
Sample Diluent (5X), 20 ml (yellow color)	3214
Wash buffer (50X), 20 ml	3210-WB
Anti-hIgG HRP Conjugate, 15 ml (light red)	3210-EC
HRP Substrate Solution (TMB) , 15 ml	3210-TM
Stop Solution, 15 ml	3210-SS
Complete Instruction Manual, M-3210-SSA	1

Rheumatoid autoimmune diseases are often associated with the occurrence of autoantibodies against several nuclear or cytoplasmatic antigens. In patients with Sjögren´s syndrome antibodies against SS-A and SS-B often occur in combination. Anti-SS-A antibodies pass the placenta and may cause the development of SLE in neonates. Each class of immunoglobulins causes a specific immunofluorescence pattern. Basically immunofluorescence titers correlate with the quantitation of IgG antibodies but the concentrations may vary considerably within each titer. Quantitation of IgG class antibodies extensively correlates with the disease activity. This makes quantitative ELISA tests superior to immunofluorescence using Hep2 cells.

Today the best investigated immunoreactive antigens are double-stranded DNA (dsDNA), single stranded DNA (ssDNA), Sm (Smith), sn-RNP (small nuclear ribonucleoprotein particles), the complex RNP/Sm which is stabilized by ribonucleic acid as well as SS-A (Ro) and SS-B (La). The antigen Scl 70, a 70 kD molecular weight protein is associated with scleroderma. In rheumatoid autoimmune diseases various profiles of autoantibodies to these antigens can be detected. They are related with high incidence to active and inactive systemic lupus erythematosus, mixed connective tissue diseases (Sharp syndrome), rheumatoid arthritis, Sjögren´s syndrome, scleroderma, photosensitive dermatitis and drug-induced lupus. In lupus patients typically anti-dsDNA antibodies can be detected. Patients without these antibodies very often show anti-ssDNA antibodies and anti-SS-A and anti-SS-B are present. A strong correlation between antibody concentration and severity of the disease has been observed with higher antibody concentrations in active phases of the disease. Thus quantitation is more informative compared to simple titering by immunofluorescence. Most of these parameters are not specific for a single disease but they occur in various combinations. The pattern of different antibody combinations and their concentration together with the whole clinical picture of the patient are helpful diagnostic tools in the assessment of rheumatoid autoimmune diseases.

Anti-SSA IgG ELISA provides a rapid quantitative measurement of Anti-SSA IgG in serum to further investigate the presence of specific autoantibodies. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of systemic lupus erythematosus (SLE) and Sjögren´s syndrome (SS).

Intra-Assay

Intra-Assay		
Sample	Mean (u/ml0	CV (%)
1	32.2	2.7
2	73.2	2.6
3	134	3.6

Inter-Assay		
Sample	Mean (u/ml0	CV (%)
1	33.8	6.4
2	71.3	6.2
3	133.1	1.1

Sensitivity

The lower detection limit for Anti-SS-A has been determined at 1.0 U/ml.

Specificity

The microplate is coated with SS-A highly purified by affinity chromatography. The Anti-SS-A test kit is specific only for autoantibodies directed to SS-A. No cross reactivities to the other ENA antigens have been observed.

Calibration

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

LIMITATIONS OF PROCEDURE

The Anti-SS-A IgG ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated. A negative Anti-SS-A result does not rule out the presence of SLE or SS.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (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.

References: Forelich H (1990) J. Rheumatol. 17, 192-200; Slobbe RI (1991) Clin. Exp. Immunol. 86, 99-105;

Interpretation of results

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

	Anti-SS-A [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-SS-A. The values above should be regarded as guidelines only.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high IgG-antibody concentrations were diluted with sample buffer and assayed in the Anti-SS-A kit.Example:

Sample	Dilution	Observed (u/ml)	Expected (u/ml)	O/E
1	1:100	139.2		
	1:200	67.9	69.5	97%
	1:400	33.0	34.8	95%
	1:800	17.2	17.4	99%
2	1:100	161.6		
	1:200	70.6	80.8	87%
	1:400	39.2	40.4	97%
	1:800	20.0	20.2	99%

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of four 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 5 determinations of each sample:

Automation

The Anti-SS-A ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

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 fulfilled, the results are invalid and the test should be repeated.

PRINCIPLE OF THE TEST

Anti-SSA IgG ELISA kit is based on binding of anti-SSA from serum samples to highly purified SSA antigen immobilized on microtiter wells. After a washing step, goat anti-human IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of anti-SSA 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 anti-SSA IgG in samples is calculated using the reference standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The ADI's ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Endpoint Cutoff and Positive 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).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not 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.

Preparation of the reagent:

Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter). **s**tore at 4oC.
Sample buffer (1:5) with distilled water (20 ml stock in total of 100-ml). **s**tore at 4oC for 30-day or until the expiration date printed on the label.

REAGENT PREPARATION FOR THE ASSAY

1. Dilute wash buffer 1:50 (20 ml stock in 980 ml water) and store at 4oC.
2. Dilute sample diluent 1:5 (20 ml stock in 80-ml water) and store at 4oC.
3. Dilute all samples to be tested 1:100 with sample diluent (10 ul sample in 990 ul of diluent or 5 ul sample in 495 ul diluent).
4. Bring all reagents and samples to room temperature (25-30oC)

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 12 months from the date of shipping under appropriate storage conditions.

Do not contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

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

1. Label, and secure the microtiter well strips to be used on the plate. **Dilute** samples (1:100) in 1x sample diluent. Controls provided in the kit are already pre-diluted. Dilute wash buffer (1:50) with distilled water (50 ml stock in total of 1-liter).
2. Pipet **100 ul of sample** diluent (for use as blanks), pre-*diluted* negative, positive controls, and *diluted* serum samples into appropriate wells in *duplicate*. Mix gently for 5-10 seconds, cover the plate and incubate for **30 minutes** at room temp (24-28oC).
3. Aspirate and **wash the wells 3 times** with 300 ul 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 values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **100 ul of enzyme conjugate** into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp.
5. Aspirate and wash the wells 4 times as above.
6. Dispense **100 ul TMB substrate per well**. Mix gently for 5 seconds. Cover the plate and incubate at room temp in the dark. for **15 minutes**. **Blue color** develops in positive wells.
7. Stop the reaction by adding **100 ul** of stopping solution to all wells at the same timed intervals . Mix gently for 5-10 seconds. **Blue color turns yellow**. 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. 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.

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 fulfilled, the results are invalid and the test should be repeated.

Calculation of results

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

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.

Typical Values of the standards

	Stds/samples	Mean OD450	Calculated Conc u/ml
A1/A2	0.00 u/ml	0.02	
B1/B2	12 u/ml	0.17	
C1/C2	25 u/ml	0.36	
D1/D2	50 u/ml	0.65	
E1/E2	100 u/ml	1.17	
F1/F2	200 u/ml	1.79	
S1/S2		0.63	49.58