

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

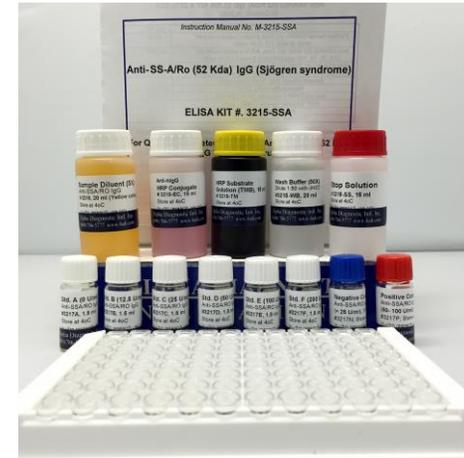
- | | | | |
|---------------------|---|--------------|---------------------------|
| #0010 | Human Leptin | | |
| #200-120-AGH | Human globular Adiponectin (gAcrp30) | | |
| #0700 | Human Sex Hormone Binding Glob (SHBG) | | |
| #0900 | Human IGF-Binding Protein 1 (IGFBP1) | | |
| #1000 | Human C-Reactive 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 Pregnenolone | #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-3215-SSA

Anti-SS-A/Ro (52 Kda) IgG (Sjögren syndrome)

ELISA KIT #. 3215-SSA

For Quantitative Determination of Anti-SS-A/Ro (52 Kda IgG In Human Serum



For In Vitro Research Use Only (RUO)



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Anti SSA/RO (52 KDA) IgG ELISA KIT # 3215-SSA

Kit Contents (96 tests):

Components	Cat. No.
Purified human SSA/Ro (52 Kda) antigen coated microwell strips (8 wells x12 or 96 wells)	3216
Anti-SSA/RO (52 KDA) 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	3217A-F
Anti-SSA/RO (52 KDA) IgG Negative Control (1.5 ml each)	3217N
Anti-SSA/RO (52 KDA) IgG Positive Control (1.5 ml each)	3217P
Sample Diluent (5X) , 20 ml (yellow color)	3218
Wash buffer (50X) , 20 ml	3215-WB
Anti-hIgG HRP Conjugate , 15 ml (light red)	3215-EC
HRP Substrate Solution (TMB), 15 ml	3215-TM
Stop Solution, 15 ml	3215-SS
Complete Instruction Manual	M-3215

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

1. True antinuclear antigens (ANA): dsDNA, ssDNA, histones, nucleolic RNA and DNP
2. Extractable nuclear antigens: Sm (Smith antigen), n-RNP, Scl-70 and PM-1
3. Cytoplasmic antigens: SS-A (Ro)*, SS-B (La)* and Jo-1 * SS-A (Ro) and SS-B (La) are co-localized in cytoplasm and nucleus

Today the best investigated immunoreactive antigens are double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), Sm (Smith antigen), 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 kDa molecular weight protein is associated with scleroderma. In rheumatoid autoimmune diseases various profiles of autoantibodies to these antigens can be detected. In a high incidence they are related 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 titrating by immunofluorescence. Measurement of anti-ssDNA provides additional information regarding antibody specificity and activity. Except in chronic inflammatory processes anti-ssDNA antibodies are not found in healthy subjects.

Sjögren syndrome type A antigen (SS-A) also known as Lupus Ro protein is a protein that in humans is encoded by the SSA gene. Anti-SS-A autoantibodies (also called anti-Ro, or the combination anti-SSA/Ro or anti Ro/SSA autoantibodies) are anti-nuclear autoantibodies that are associated with among many autoimmune diseases, such as systemic lupus erythematosus (SLE), SS/SLE overlap syndrome, subacute cutaneous LE (SCLE), neonatal lupus and primary biliary cirrhosis. Also, they are often present in Sjögren's syndrome. Two proteins associated with Sjögren's Syndrome were independently described as antigens A and B, but are now known to be identical to Ro and La respectively. i.e. SS-A = Ro and SS-B = La. The Ro/La system is considered as a heterogeneous antigenic complex, constituted by three different proteins (**52 kDa Ro, 60 kDa Ro and La**) and four small RNAs particles. Anti-Ro/SSA are the most prevalent specificity among many autoimmune diseases, such as systemic lupus erythematosus (SLE), SS/SLE overlap syndrome, subacute cutaneous LE (SCLE), neonatal lupus and

Intra-Assay

Intra-Assay		
Sample	Mean (u/ml)	CV (%)
1	16.1	2.7
2	36.6	2.6
3	67.0	3.6

Inter-Assay		
Sample	Mean (u/ml)	CV (%)
1	16.9	6.4
2	35.7	6.2
3	65.1	1.1

Sensitivity

The lower detection limit for Anti-SSA/RO (52 KDA) has been determined at 0.5 U/ml.

Specificity

The microplate is coated with SSA/RO (52 KDA) highly purified by affinity chromatography. The Anti-SSA/RO (52 KDA) test kit is specific only for autoantibodies directed to SS-A/Ro (52 Kda). No cross reactivities to the other SSA/Ro (60 Kda) 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-SSA/RO (52 KDA) 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:	< 25
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.

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.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high IgG-antibody concentrations were diluted with sample buffer and assayed in the Anti-SSA/RO (52 KDA) 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%
2	1:800	17.2	17.4	99%
	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-SSA/RO (52 KDA) ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

primary biliary cirrhosis. In contrast, anti-La/SSB is more associated with Sjögren's syndrome (SS). Human Anti-SS-A (60/52kda) IgG ELISA is an indirect solid phase enzyme-linked immunosorbent assay (ELISA). It is designed for the quantitative measurement of IgG class autoantibodies directed against the extractable nuclear antigens SS-A (Ro) (autoantibodies) in human serum or plasma. Anti-SSA/60Kda and Anti-SSA/52kda ELISA use recombinant purified protein so and the antibodies detected are specific to SSA/60kda or SSA/52kda. This kit does not measure other Ig's subclasses such as IgM, IgA or IgE or antibodies to other ENA/ANA

In patients with Sjögren's syndrome antibodies against SS-A and SS-B often occur in combination. Due to the strong association of SS-A and SS-B antibodies to the HLA-DR3 and DR2 phenotypes a genetic predisposition is suspected. The anti SS-A protein passes the placenta and may cause the development of SLE in neonates. Immunoreactive proteins may occur in various combinations and bind also to 'host proteins' of viral origin. They induce synthesis of polyclonal autoantibodies, of the IgG, IgM and IgA class. Especially for mixed connective tissue diseases a relation to viral infections by EBV (Epstein-Barr virus) is indicated. Each class of immunoglobulins causes a specific immunofluorescent 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 diseases' activity. This makes it superior to immunofluorescence using Hep2 cells. The IF with Crithidia lucilliae sometimes results in deviating values. Most of these parameters are not specific for just one 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.

The following graph gives brief information on the complexity of autoimmune diseases, occurring antibodies. It is not designed as a diagnostic schedule or program for ongoing diagnostic profiles.

Diseases	Anti-								
	dsDNA	ssDNA	Histon	SS-A (Ro)	SSB (La)	Sm	RNP/Sm	Scl-70	Jo-1
Systemic lupus erythematosus (SLE)	>90	>90	30-50	10-30	30-50	10-30			
Drug induced lupus (LE)		30-50	50-90						
Sharp syndrome / mixed connective tissue diseases	10-30	10-30					> 90		
Rheumatoid arthritis		30-50	30-50	10-30					
Sjögren's syndrome	10-30	10-30		> 90	> 90				
Scleroderma	10-30	10-30		10-30				> 90	
Photosensitive dermatitis dermatomyositis	10-30	10-30							50-90

Anti-SSA/Ro (52 Kda) IgG ELISA provides a rapid quantitative measurement of Anti-SSA/Ro (52 kda) 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).

PRINCIPLE OF THE TEST

Anti-SSA/RO (52 Kda) IgG ELISA kit is based on binding of anti-SSA/RO (52 Kda) from serum samples to highly purified SSA/Ro 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/RO (52 Kda) 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/RO (52 Kda IgG in samples is calculated using the reference standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 μ l) 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 cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples.

Preparation of the reagent:

Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter). store at 4oC.

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

STORAGE AND STABILITY

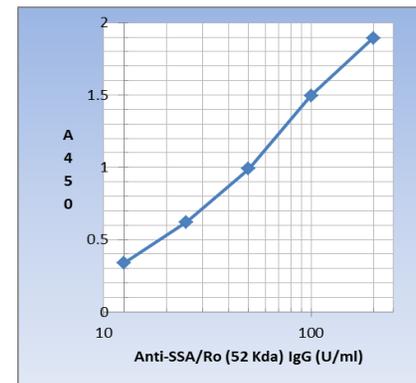
The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6-12 months from the date of shipping under appropriate storage conditions. .

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. **Standards & Controls provided in the kit are ready-to-use.** Dilute wash buffer (1:50) with distilled water (50 ml stock in total of 1-liter).
2. Pipet **100 μ l** of standards, controls and prediluted patient samples in 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 μ l** 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 μ l 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 **3 times** as above.
6. Dispense **100 μ l 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 μ l** 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.

Calculation of results

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 (do not use this graph for sample calculation)

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