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User's Manual

Human anti-SSA 60 Ro IgG ELISA Kit

REF **DEIA-NS2405-4**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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 research use only (RUO).

General Description

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 druginduced 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.

Principles of Testing

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.

Reagents And Materials Provided

1. Nuclear antigen coated microwell strips (96 wells),
2. 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
3. Anti-SSA IgG Negative Control (1.5 ml each)
4. Anti-SSA IgG Positive Control (1.5 ml each)
5. Sample Diluent (5X), 20 ml (yellow color)
6. Wash buffer (50X), 20 ml
7. Anti-hIgG HRP Conjugate, 15 ml (light red)
8. HRP Substrate Solution (TMB) , 15 ml
9. Stop Solution, 15 ml
10. Complete Instruction Manual

Materials Required But Not Supplied

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

Storage

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.

Specimen Collection And Preparation

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.

Reagent Preparation

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

Assay 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 1× 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 µl of sample diluent (for use as blanks), ready-to-use 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 (24-28°C).
3. 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 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 µl 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

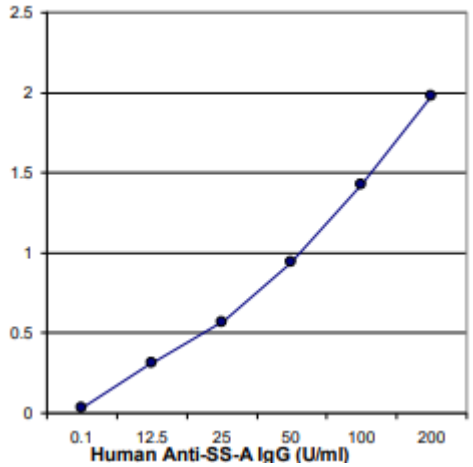
For Anti-SS-A a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

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



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.

Calibration:

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

Precision

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.

Interferences

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.

Precautions

The 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.

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

Limitations

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-SSA result does not rule out the presence of SLE or SS.