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User's Manual

SS-A (Ro) IgG ELISA Kit

REF **DEIA4222**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The SS-A (Ro) IgG ELISA Kit is intended for the detection of IgG antibody to SSA in human serum or plasma.

General Description

Systemic autoimmune disease is characterized by the presence of circulating auto-antibodies directed to a wide variety of cellular antigens. Systemic lupus erythematosis (SLE), commonly referred to as Lupus is the best known of these diseases. Other possible connective tissue diseases include mixed connective tissue disease (MCTD), Sjogren syndrome, sclerodema, and polymyositis/dermatomyositis. The majority can be diagnosed by clinical presentation and their antibody profiles to the various antigens involved, which include dsDNA, SM, RNP, SSA, SSB, Scl-70, Jo1 and Histones. Therefore, immunoassays for autoantibodies are useful for diagnostic and prognostic evaluations of autoimmune disease. SSA (Ro) antigen (60kd) and 52 kd polypeptides complexed with Ro RNAs are detected in about 75% of primary and secondary Sjögren syndrome, In>90% of subacute cutaneous lupus and in the vasculitis-associated Sjögren syndrome, SS-A autoantibodies are present and are accompanied in ~50% by SS-B/La autoantibodies. The coexistence of SSA and SSB autoantibodies probably reflects the presence of these polypeptides on the same particle and the spreading among autoimmunity to these self antigens.

Principles of Testing

Diluted serum sample is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

Reagents And Materials Provided

1. Microwells coated with SSA antigen 12x8x1
2. Sample Diluent: 1 bottle (ready to use) 22 ml
3. Calibrator: 1 Vial (ready to use) 1.5 ml
4. Positive Control: 1 vial (ready to use) 1.5 ml
5. Negative Control: 1 vial (ready to use) 1.5 ml
6. Enzyme conjugate: 1 bottle (ready to use) 12 ml
7. TMB Substrate: 1 bottle (ready to use) 12 ml
8. Stop Solution: 1 bottle (ready to use) 12 ml
9. Wash concentrate 20×: 1 bottle 25 ml

Materials Required But Not Supplied

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

Storage

Store the kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose test reagents to heat, sun or strong light.

Specimen Collection And Preparation

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

Reagent Preparation

Prepare 1× Wash buffer by adding the contents of the bottle (25 mL, 20×) to 475 mL of distilled or deionized water. Store at room temperature (20-25°C).

Assay Procedure

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µL of the sample to 200 µL of sample diluent. Mix well.
3. Dispense 100 µL of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µL sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µL of 1× wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µL of 1× wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µL of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µL of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

Quality Control

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

Calculation

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD × Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.
4. Example of typical results:

   Calibrator mean OD = 0.8

   Calibrator Factor (CF) = 0.5

   Cut-off Value = 0.8 x 0.5= 0.400

   Positive control O.D. = 1.2

   Ab Index = 1.2 / 0.4 = 3

   Sample O.D. = 1.6

   Ab Index = 1.6 / 0.4 = 4.0

Performance Characteristics

Interpretation

The following is intended as a guide to interpretation of SSA test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

<0.9 No detectable antibody to SSA by ELISA.

0.9-1.1 Borderline positive. Follow-up testing is recommended.

>1.1 Detectable antibody to SSA by ELISA.

Precision

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.61	0.101	6.2
2	16	0.88	0.062	6.8
3	16	0.2	0.017	8.5

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.78	0.125	7.0
2	10	0.95	0.088	9.2
3	10	0.22	0.024	10.9

Precautions

1. This kit is designed for research use only.
2. Potential biohazardous materials:

   The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

Limitations

Lipemic or hemolyzed samples may cause erroneous results.