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Product Information

SSA Autoantibody ELISA

Catalog Number **SE120117**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Systemic autoimmune disease is characterized by the presence of circulating autoantibodies 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 (60 kDa) and 52 kDa polypeptides complexed with Ro RNAs are detected in ~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.

The SSA Autoantibody ELISA is intended for the detection of IgG antibody to SSA in human serum or plasma. Diluted serum 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 oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

Components

Materials Provided	96 Tests
Microwells coated with SSA antigen	12 x 8 x 1
Sample Diluent: 1 bottle (ready to use)	22 mL
Calibrator: 1 vial (ready to use)	1 mL
Positive Control: 1 vial (ready to use)	1 mL
Negative Control: 1 vial (ready to use)	1 mL
Enzyme conjugate: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
Wash concentrate 20x: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample 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.

20x Wash Buffer Concentrate

Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

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.

Lipemic or hemolyzed samples may cause erroneous results

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

1. The O.D. of the Calibrator should be >0.250.
2. The Ab index for Negative control should be <0.9.
3. The Ab Index for Positive control should be >1.2.

Bring all specimens and kit reagents to room temperature (18–26 °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 21-fold 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 100 μL of 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 1x Wash buffer. Blot on absorbent 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 1x Wash buffer. Blot on absorbent 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 minutes. A dual wavelength is recommended with reference filter of 600–650 nm.

Results

Calculations

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. Calculate the cut-off value: Calibrator OD x 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.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 × 0.5 = 0.400
Positive control O.D. = 1.2
Ab Index = 1.2/0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6/0.4 = 4.0

Interpretation

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

<0.9 – No detectable antibody to SSA by ELISA
0.9–1.1 – Borderline positive. Follow-up testing is recommend if clinically indicated.
>1.1 – Detectable antibody to SSA by ELISA

References

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