

Product Information

SSB Autoantibody ELISA kit

Catalog Number: EA100924

Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The SSB (La) IgG ELISA Kit is intended for the detection of IgG antibody to SSB in human serum or plasma.

Background

Systemic autoimmune disease is characterized by the presence of circulating auto-antibodies directed to a wide variety of cellular antigens. Systemic lupus erythematosus (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, scleroderma, 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. The 48 kd phosphoprotein known as SSB (La) is a transcription termination factor for RNA polymerase III. SSB shuttles between nucleus and cytoplasm and exists both free and as a component of the SSA/SSB ribonucleoprotein cytoplasmic particle. Autoantibodies to SSB are detected by ELISA in ~70-90% of primary and ~50% of secondary Sjögren syndrome as well as in ~25% of SLE and ~80% of subacute cutaneous lupus and in the majority of infants with complete heart block. SSB autoantibodies are found only in sera determined to contain SSA autoantibodies by a sensitive method; this probably reflects the association of the SS-A and SS-B antigens in a macromolecular complex.

Principle of the Test

Diluted patient 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 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.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with SSB antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Enzyme conjugate: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12 ml

5. Calibrator: 1 Vial (ready to use)	1 ml
6. Positive Control: 1 vial (ready to use)	1 ml
7. Negative Control: 1 vial (ready to use)	1 ml
8. Stop Solution: 1 bottle (ready to use)	12 ml
9. Wash concentrate 20X: 1 bottle	25 ml

Materials 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 450nm
5. Absorbance paper or paper towel

Disclaimer

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

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

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
 - The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
 - It is recommended that standards, control and serum samples be run in duplicate
 - Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities
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 100 µl 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 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 1X 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.

Calculation of Results

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 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 a Standard Curve

Calibrator mean OD = 0.8

Calibrator Factor (CF) = 0.5

Cut-off Value = $0.8 \times 0.5 = 0.400$

Positive control O.D. = 1.2

Ab Index = $1.2 / 0.4 = 3$

Patient sample O.D. = 1.6

Ab Index = $1.6 / 0.4 = 4.0$

Quality Control

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

1. If 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 be greater than 1.2.

Interpretation

The following is intended as a guide to interpretation of SSB antibody 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 SSB by ELISA
- 0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
- >1.1 Detectable antibody to SSB by ELISA

References

1. Peek R, van Venrooij WJ, Simons F, Pruijn G. The SS-A/SS-B autoantigenic complex: localization and assembly. *Clin Exp Rheum*, 1994;12:S15-S18.
2. Keech CL, Gordon TP, Reynolds P, McCluskey J. Expression and functional conservation of the human La (SS-B) autoantigen in murine cell lines. *J Autoimmun*, 1993;6:543-55.
3. Topfer F, Gordon T, McCluskey J. Intra- and intermolecular spreading of autoimmunity involving the nuclear self- antigens La (SS-B) and Ro (SS-A). *Proc Natl Acad Sci USA*, 1995;92:875-9.
4. Keech CL, McCluskey J, Gordon TP. SS-B (La) Autoantibodies. In: Peter JB, Shoenfeld Y, editors. *Autoantibodies*. Amsterdam: Elsevier 1996:789-97.
5. Owe-Young RA, Horn S, Edmonds JP, Sturgess AD. Use of a molecularly cloned human SS-B antigen to detect anti-SS-B antibodies. *J Autoimmun* 1992;5:351-61.
6. Harley JB. Autoantibodies in Sjögren's syndrome. *J Autoimmun* 1989;2:283-394.
7. levels to 52 kd Ro (SS-A) and La (SS-B) in complete congenital heart block. *J Autoimmun* 1995;8:675-84.

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8. Beer RG, Rischmueller M, Coates T, Purcell AW, Keech CL, McCluskey J, Gordon TP. Nonprecipitating anti- La(SS-B) autoantibodies in primary Sjögren's syndrome. Clin Immunol Immunopathol 1996;79:314-8.

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