

**REFERENCES**

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## SSB Autoantibody ELISA

Catalog No. SB041G (96 Tests)

**INTENDED USE**

The Calbiotech, Inc. SSB (La) IgG ELISA Kit is intended for the detection of IgG antibody to SSB in human serum or plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

**SUMMARY AND EXPLANATION**

Anti-SSB (La) antibodies target a ribonucleoprotein involved in RNA metabolism. These autoantibodies are studied in the context of systemic autoimmune diseases, immune dysregulation, and epitope mapping in research laboratories.

**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.

MATERIALS PROVIDED	96 TESTS
1. Microwells coated with SSB antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 mL
3. Calibrator: 1 Vial (ready to use)	1 mL
4. Positive Control: 1 vial (ready to use)	1 mL
5. Negative Control: 1 vial (ready to use)	1 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 20X: 1 bottle	25 mL

**MATERIALS 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
6. Graph paper

**STORAGE AND STABILITY**

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

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**WARNINGS AND PRECAUTIONS**

1. 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.
2. 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.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. 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.

**SPECIMEN COLLECTION AND HANDLING**

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

**REAGENT PREPARATION**

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 (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 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. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel
6. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
7. Add 100 µl of stop solution.
8. 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.

**LIMITATIONS OF THE TEST**

1. Lipemic or hemolyzed samples may cause erroneous results.