9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989

SSB IgG ELISA Kit

Name	SSB IgG ELISA Kit
INTENDED USE	The Calbiotech, Inc. (CBI) SSB (La) IgG ELISA Kit is intended for the detection of IgG antibody to SSB in human serum or plasma.
SUMMARY AND EXPLANATION	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. 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.

MATERIALS PROVIDED	96 Tests
Microwells coated with SSB antigen	12x8x1
Sample Diluent: 1 bottle (ready to use)	22 ml
Calibrator: 1 Vial (ready to use)	1ml
Positive Control: 1 vial (ready to use)	1ml
Negative Control: 1 vial (ready to use)	1ml
Enzyme conjugate: 1 bottle (ready to use)	12ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
Wash concentrate 20X: 1 bottle	25ml

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

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.

9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989

WARNINGS AND PRECAUTIONS	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. 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. 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	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 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. Place the desired number of coated strips into the holder. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 l of the sample to 200 l of sample diluent. Mix well.

9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989

	Dispense 100 l of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100l 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. Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel. Dispense 100 l 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 l of 1X wash buffer. Blot on absorbance paper or paper towel Dispense 100 l of TMB substrate and incubate for 10 minutes at room temperature. Add 100 l of stop solution. 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	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. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF). 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 x 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
LIMITATIONS OF THE TEST	The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the

patient's history, physical findings and other diagnostic procedures.

Lipemic or hemolyzed samples may cause erroneous results.

QUALITY CONTROL

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

The O.D. of the Calibrator should be greater than 0.250.

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.

INTERPRETATION

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

Antibody Index Interpretation

1.1 Detectable antibody to SSB by ELISA

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

117 patient sera were tested by this SSB IgG ELISA and a reference ELISA method. 16 sera were positive and 95 were negative by both methods (95% agreement). The results are summarized below:

	SSB IgG ELISA	SSB IgG ELISA	SSB IgG ELISA
	+		Total
Reference ELISA Kit +	16	3	19
_	3	95	98
Total	19	98	117

Precision

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %

1	16	1.45	0.095	6.5
2	16	0.76	0.056	7.3
3	16	0.21	0.018	8.6

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.54	0.126	8.2
2	10	0.82	0.91	11.1
3	10	0.23	0.026	11.3

REFERENCES

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.

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.

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.

Keech CL, McCluskey J, Gordon TP. SS-B (La) Autoantibodies. In: Peter JB, Shoenfeld Y, editors. Autoantibodies. Amsterdam: Elsevier 1996:789-97.

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.

Harley JB. Autoantibodies in Sjögren's syndrome. J Autoimmu 1989;2:283-394.

levels to 52 kd Ro (SS-A) and La (SS-B) in complete congenital heart block. J Autoimmun 1995;8:675-84.

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.

9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989