

**CALDON BIOTECH INC.**  
**Double Stranded DNA**  
**(dsDNA) IgG**  
**ELISA**

Catalog No. DD037G  
 (96 tests)

**NAME AND INTENDED USE**

The CALDON BIOTECH INC (CBI) dsDNA IgG ELISA is intended for the use in the evaluation of patients with suspected autoimmune diseases.

**SUMMARY AND EXPLANATION OF THE TEST**

Anti-dsDNA is present in 50 % to 70% of patients with systemic lupus erythematosus (SLE). Circulating DNA/anti-DNA immune complexes are considered to play a part in the pathogenesis of SLE. The presence of anti-dsDNA is one of the diagnostic criteria for SLE. IgG antibodies to dsDNA are considered clinically most useful for the diagnosis and management of SLE. Antibodies to single stranded DNA (ssDNA) and IgM antibodies to DNA are found in a number of other connective diseases, liver diseases, as well as in some normal individuals. ELISA is the method of choice for the screening of anti-dsDNA in patients with suspected SLE.

**PRINCIPLE OF THE TEST**

Diluted patient serum is added to wells coated with purified dsDNA antigen. 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 specific antibody in the sample.

**MATERIALS PROVIDED**

- Microwell Strips: dsDNA coated 96 well plate (12 x 8 x 1 wells).
- Wash Concentrate: 1 bottle (50 mL) 20X.
- Sample Diluent: 1 bottle (22 mL). Ready to use.
- Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
- TMB Substrate: 1 bottle (12 mL). Ready to use.
- Calibrator: 1 vial (1.50 mL). Ready to use.

- Positive Control: 1 vial (1.50 mL). Ready to use.
- Negative Control: 1 vial (1.50 mL). Ready to use.
- Stop Solution: 1N H<sub>2</sub>SO<sub>4</sub>; 1 bottle (12 mL). Ready to use.

**STORAGE AND STABILITY**

- 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 during storage or usage.

**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. This product contains components 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 of serum sample.

**PREPARATION FOR ASSAY**

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

2. Prepare washing buffer by adding the contents of the bottle (50 mL, 20X Wash concentrate) to 950 mL of distilled or deionized water in one-liter container. Store at room temperature.

**ASSAY PROCEDURE**

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 control 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. Discard liquid from all wells. Wash each well three times with 300 µL wash buffer.
5. Dispense 100 µL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Discard liquid from all wells. Wash each well three times with 300 µL wash buffer.
7. Dispense 100 µL of TMB substrate solution and incubate for 10 minutes at room temperature.
8. Add 100 µL of 1N H<sub>2</sub>SO<sub>4</sub> to stop reaction.
9. Read O.D. within 30 min at 450 nm with a microwell reader.

**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 cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the mean values 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

**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.

- The Ab Index for Positive control should be greater than 1.2.

**INTERPRETATION**

The following is intended as a guide to interpretation of CBI dsDNA 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 dsDNA.
- 0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.
- >1.1 Indicative of autoimmune diseases.

**LIMITATIONS OF THE TEST**

1. 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.
2. Lipemic or hemolyzed samples may cause erroneous results.

**PERFORMANCE CHARACTERISTICS**

**1. Sensitivity and Specificity**

142 sera from patients with suspected autoimmune diseases were tested by both CBI dsDNA ELISA and a reference ELISA method. 17 were positive and 120 were negative by both methods (96% agreement).  
 The results are summarized below:

	CBI dsDNA antibody ELISA		
	+	-	Total
Reference ELISA +	17	3	20
Kit -	2	120	122
Total	19	123	142

**2. Precision  
 Intra-Assay Study**

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

1	16	1.76	0.09	5.11
2	16	0.81	0.05	6.20
3	16	0.24	0.02	8.33

### Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	10	1.49	0.11	7.38
2	10	1.1	0.09	8.18
3	10	0.28	0.03	10.7

### REFERENCES

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