

CALDON BIOTECH INC. ANA Screen ELISA

Catalog No. EANA 001
(96 tests)

NAME AND INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

Antinuclear antibodies (ANA) are frequently present in patients with systemic lupus erythematosus (SLE) and, less commonly, in other autoimmune diseases Rheumatoid arthritis, Collagen vascular diseases, chronic liver diseases and systemic sclerosis (scleroderma). ANA bind to several nuclear antigens including DsDNA, SSDNA, RNP, Sm, SSA and SSB. ANA frequency increases with age in apparently healthy people, especially women after the age of 45 years. ANA ELISA is widely used as a screening procedure for different autoimmune diseases.

PRINCIPLE OF THE TEST

Diluted patient serum is added to wells coated with purified ANA antigen. ANA 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

1. Microwell Strips: Nuclear Antigen coated wells (12 x 8 x 1 wells)
2. Sample Diluent: Green color. 1 Bottle (22 mL). Ready to use
3. HRP Enzyme Conjugate: Red color. 1 Bottle (12 mL). Ready to use.
4. TMB Substrate: 1 Bottle (12 mL). Ready to use.
5. Stop Solution: 1N H₂SO₄; 1 Bottle (12 mL). Ready to use.
6. Calibrator: Yellow Cap. (0.150 mL/vial).
7. Positive Control: Red Cap. (0.150 mL /vial).
8. Negative Control: Blue Cap. (0.150 mL /vial).

9. Wash Concentrate: 1 Bottle (50 mL) 20X Concentrate.

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 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, as 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. Prepare 1:21 dilution of test samples, negative control, positive control, and calibrator 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. Remove liquid from all wells. Repeat washing three times with washing buffer.
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. Repeat washing three times with washing buffer.
7. Dispense 100 µL of TMB substrate solution and incubate for 10 minutes at room temperature.
8. Add 100 µL of 1N H₂SO₄ to stop reaction.
9. Read O.D. on ELISA reader at 450 nm within 30 minutes after adding stop solution.

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 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:

- If 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 ANA IgG 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 ANA IgG by ELISA.

0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.

>1.1 Indicative of autoimmune disorder.

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 patients history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

354 patient sera were tested by both CBI ELISA and a reference ELISA methods. 148 sera were positive and 188 sera were negative by both methods. The agreement between the two methods was 95% (336/354). The results are summarized below:

	CBI ANA IgG ELISA			Total
	+	-		
Reference ELISA +	148	6	4	158
Kit +	2	0	0	2
-	6	0	188	194
Total	156	6	192	354

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2. Precision Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	16	1.41	0.06	4.22
2	16	0.82	0.02	2.6
3	16	0.29	0.03	9.48

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	10	1.15	0.09	7.43
2	10	0.81	0.1	11.8
3	10	0.29	0.03	9.48

REFERENCES

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