dsDNA IgG ELISA Kit

Catalog Number KA0944

96 assays

Version: 05

Intended for research use only

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Table of Contents

Introduction

Intended Use

The dsDNA IgG ELISA Kit is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG antibody to dsDNA in human serum or plasma.

Background

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 Assay

Diluted 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.

General Information

Materials Supplied

List of component

Component	Amount
Microwells coated with dsDNA antigen	96 (12 x 8) wells
Sample Diluent (ready to use)	22 mL
Enzyme conjugate (ready to use)	12 mL
TMB Substrate (ready to use)	12 mL
Calibrator (ready to use)	1 mL
Positive Control (ready to use)	1 mL
Negative Control (ready to use)	1 mL
Stop Solution (ready to use)	12 mL
Wash concentrate 20X	25 mL

Storage Instruction

- ✓ 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.

Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450 nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

Precautions for Use

- ✓ 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.
- 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.

✓ Limitations of the Test

- Lipemic or hemolyzed samples may cause erroneous results.

Assay Protocol

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).

Sample 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.

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 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 solution 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.

Data Analysis

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 mean 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

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. 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 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 by ELISA.
0.9-1.1	Borderline positive. Follow-up testing is recommend.
>1.1	Detectable antibody to dsDNA by ELISA.

Performance Characteristics

✓ Sensitivity and Specificity

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

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

✓ 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

Resources

Reference

1. Wong KH; Lawton JW; Cheng SK; Lee SS; Lau CS. Measurement of anti-dsDNA: a comparative study of two ELISA and the Crithidia assay. Pathology 1998; 30(1):57-61.
2. Bootsma H; Spronk PE; Ter Borg EJ; Hummel EJ; de Boer G; Limburg PC; Kallenberg CG. The predictive value of fluctuations in IgM and IgG class anti-dsDNA antibodies for relapses in systemic lupus erythematosus. A prospective long-term observation. Ann Rheum Dis 1997; 56(11):661-6.
3. Takeuchi Y; Ishikawa O; Miyachi Y. The comparative study of anti-double stranded DNA antibody levels measured by radioimmunoassay and enzyme-linked immunosorbent assay in systemic lupus erythematosus. J Dermatol 1997; 24(5):297-300.
4. Batini´c D; Bo¨zi´cevi´c M; Krstulovi´c A; Bosni´c D; Senti´c M; Markeljevi´c J; Malenica B; Cike¨s N; Maru¨si´c M. Binding of anti-double stranded (ds) DNA-positive sera to denatured (d) DNA and synthetic poly[dA-dT] x poly[dA-dT] double stranded copolymer in an ELISA format. Eur J Clin Chem Clin Biochem 1996; 34(4):343-7.
5. Tomer Y; Viegas OA; Swissa M; Koh SC; Shoenfeld Y. Levels of lupus autoantibodies in pregnant SLE patients: correlations with disease activity and pregnancy outcome. Clin Exp Rheumatol 1996; 14(3):275-80.
6. Avina-Zubieta JA; Galindo-Rodriguez G; Kwan-Yeung L; Davis P; Russell AS. Clinical evaluation of various selected ELISA kits for the detection of anti-DNA antibodies. Lupus 1995; 4(5):370-4.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
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