

Product Information

Double Stranded DNA (dsDNA) IgG ELISA kit

Catalog Number: EA100918

Storage Temperature: 2 – 8°C

Instruction for Use

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 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 oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of specific antibody in the sample.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with dsDNA antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Enzyme conjugate: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12 ml
5. Calibrator: 1 Vial (ready to use)	1 ml
6. Positive Control: 1 vial (ready to use)	1 ml
7. Negative Control: 1 vial (ready to use)	1 ml
8. Stop Solution: 1 bottle (ready to use)	12 ml
9. Wash concentrate 20X: 1 bottle	25 ml

Materials and Equipment Required but 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

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and 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.

Reagent Preparation

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C). Gently mix all reagents before use
 - The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
 - It is recommended that standards, control and serum samples be run in duplicate
 - Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities
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 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.

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

Lipemic or hemolyzed samples may cause erroneous results.

References

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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. Batinić D, Božičević M, Krstulović A, Bosnić D, Sentić M, Markeljević J, Malenica B, Čikeš N, Marušić M. Binding of anti double stranded (ds) DNA positive sera to denatured (d) and synthetic poly (dAdT) x poly(dAdT) double stranded copolymer in an ELISA format. *Eur J Clin Chem Clin Biochem* 1996;34:343-7
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.

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