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 IVD


See external label



2°C-8°C



Σ=96 tests

 REF

#2553

**MICROWELL ELISA**  
**Double Stranded DNA**  
**ANTI-dsDNA IgG ANTIBODY ELISA**

**Catalog No. 2553**

(96 tests)

**INTENDED USE**

The Diagnostic Automations Anti-dsDNA test is a quantitative enzyme immunoassay (EIA) intended to screen for the presence of dsDNA antibodies in human serum as an aid in the diagnosis of systemic lupus erythematosus (SLE).

**SUMMARY and EXPLANATION**

Antinuclear antibodies (ANAs) directed against a variety of macromolecules occur in extraordinarily high frequency in systemic rheumatic diseases. Many rheumatic diseases are characterized by the presence of one or more of these ANAs. Therefore, the identification of the specific antibody is useful in the detection and diagnosis of the disease (1).

Anti-dsDNA is present in 50% to 70% of patients with SLE (2,3). Circulating DNA/anti-DNA immune complexes are considered to play a part in the pathogenesis of SLE (3). The presence of anti-dsDNA is one of the diagnostic criteria for SLE (4). IgG antibodies to dsDNA are considered clinically most useful for the diagnosis and management of SLE (5-10). Antibodies to single stranded DNA (ssDNA) and IgM antibodies to dsDNA are found in a number of other connective diseases, liver diseases, as well as in some normal individuals (11,12). Accurate detection of anti-dsDNA is important in the diagnosis and management of SLE. EIA tests for anti-dsDNA have demonstrated greater sensitivity than standard IFA and RIA tests allowing for improved detection of low titer antibodies to dsDNA (12).

**PRINCIPLE**

Purified dsDNA is bound to microwells. The DNA retains its antigenicity and remains double stranded. Antibodies to dsDNA, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically binds to the bound patient antibodies forming a "conjugate - anti-dsDNA - dsDNA" sandwich. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end product. The intensity of the color is measured photometrically at 450nm.

## WARNINGS and PRECAUTIONS

- 1) For In vitro diagnostic use only.
- 2) Do not pipette by mouth.
- 3) Avoid contact with open skin.
- 4) Components containing human serum have tested negative for HBsAG and HIV antigens. This does not assure the absence of these antigens; sera should be considered potentially hazardous. These materials should be handled as recommended in the CDC/NIH Health manual, Biosafety in Microbiological and Biomedical Laboratories, 1984.
- 5) Sodium Azide is a toxic substance and is used in some reagents. In case of contact with eyes and skin, flush immediately with copious amounts of water. Sodium azide may react with lead and copper plumbing to form explosive metal azides. When disposing reagents flush with a large volume of water to help prevent azide build-up.
- 6) The Stop Buffer contains a dilute acid solution. Use with care to avoid contact with skin and eyes. Avoid exposure to bases, metals, or other compounds, which may react with acids. Spills should be cleaned up immediately.

## SPECIMEN COLLECTION

Collect blood aseptically in untreated tubes. Allow blood to clot, separate serum immediately. Avoid use of lipemic, hemolyzed or contaminated sera. Store sera at 2-6°C. Freeze sera at -20°C if not tested within 24 hours; avoid repeated freezing.

CAUTION: Serum samples should NOT be heat-inactivated as this may cause false positive results.

## MATERIALS REQUIRED

EIA Reader (set to 450 nm)  
Micropipettors (10µl & 100µl)  
8-channel repeating pipettor (for washing)  
Deionized (DI) Water  
Pipettes (1ml & 10ml)  
1 Liter Container (for DNA Wash Solution)  
Test Tubes (4ml & 15ml)  
Countdown timer

## RECOMMENDED MATERIALS

Automatic Washer  
100ul 8-Channel Micropipettor (for reagent delivery)  
1ml Mini-Tubes (for sample dilutions)

## MATERIALS SUPPLIED

**dsDNA Microtiter Strips:** 96 antigen-coated wells, sealed in a resealable foil pouch with desiccant. Ready to use.

**EIA Frame:** Use to hold antigen-coated wells. Retain Frame for future use.

**DNA Wash Concentrate:** To 1 liter with DI water. Save for future use.

**DNA Diluent:** 120ml. Ready to use. Use to dilute Positive Control, Positive Calibrator, Negative Control, and patient samples 1:100. Use for blanking control. Avoid unnecessary contamination; contains thimerosal as a preservative.

**HRP Anti-Human IgG** 15ml. Ready to Use.

**Anti-dsDNA Positive Control:** 0.45ml. Stabilized human plasma. Dilute 1:100 in Diluent at same time and in same manner as samples; gives positive OD reading.

**Anti-dsDNA Calibrator:** 0.45ml. Stabilized human plasma. Dilute 1:100 in Diluent at same time and in same manner as samples. Use to calculate sample's International Units (IUs).

**Anti-dsDNA Negative Control:** 0.45ml. Stabilized human plasma. Dilute 1:100 in Diluent at same time and in same manner as samples; gives a negative OD reading.

**TMB Color Reagent:** 15ml. Ready to use.

**StopBuffer:** 15ml. Use to stop color development. Contains sulfuric and hydrochloric acid. Read at a wavelength of 450 nm.

## **STABILITY and STORAGE of REAGENTS**

The kit is stabilized for ambient shipment. All kit components should be stored at 2-8°C and can be used until the expiration date printed on the labels. Once the antigen-coated well foil pouch has been opened, the wells are stable for 30 days.

## **PROCEDURE NOTES**

- 1) All materials must be at room temperature.
- 2) Do not use calibrators from different kit lots. Do not use expired reagents.
- 3) Avoid contamination of reagents, dispensing pipettes, and microtiter wells. Use new dispensing pipettes for all samples. Do not interchange caps. Always keep bottles capped when not in use. Do not reuse the microtiter wells or pipettes. Avoid pipettes contaminated with peroxidase.
- 4) All wells should be handled in the same sequence and the same manner throughout the test. The test should be performed without interruptions.
- 5) Gently and completely swirl each bottle of liquid reagent and sample before use.
- 6) Make reliable 1:100 dilutions.
- 7) Make all dilutions in uncontaminated DNA Diluent. Prepare all dilutions before starting test. Always use fresh sample dilutions.
- 8) Always run a Positive Control, a Positive Calibrator, and a Negative Control. Always blank against DNA Diluent.
- 9) Humidity affects the antigen-coated wells; do not open pouch until it reaches room temperature. Calculate the number of wells required for the current assay, remove them from the room temperature foil pouch, align them on the EIA Frame, and then add samples immediately. Unused wells should be returned immediately to the resealed foil pouch with desiccant.
- 10) Incubation times affect EIA results. Do not allow any of the controls, samples or HRP to incubate in the strip wells for more than 40 minutes. For best results, use 1ml mini-tubes to prepare sample dilutions. Transfer all solutions into wells with an 8-channel Micropipettor.
- 11) After each incubation, thoroughly wash the microtiter wells with ~200µl Wash Solution per well. Be sure to remove all liquid before proceeding to next step. Fill wells, then invert and rapidly flick away the liquid. After complete washing, blot the plate on a paper towel.
- 12) Transfer to a graduated test tube 1ml of HRP for each strip to be run. Discard excess transferred HRP.
- 13) Transfer to a graduated test tube 1ml of TMB for each strip to be run. Discard excess transferred TMB.

## **PRE-TEST SETUP INSTRUCTIONS**

- 1) Collect all reagents, samples, and dilutions necessary before starting assay.
- 2) Prepare DNA Wash Solution by diluting DNA Wash Concentrate to 1 liter with DI water. Mix thoroughly. Use to wash wells. Save for future use. Uncontaminated DNA Wash Solution is good for 14 days.
- 3) Allow Diluent to come to room temperature before use. Mix thoroughly. Use Diluent to make all dilutions. Avoid unnecessary contamination.
- 4) Assign and record wells for controls and samples.
- 5) Make 1:100 working solutions:
  - a) Dilute 10µl of patient's sera in 1.0 ml of DNA Diluent.
  - b) Dilute 10µl of anti-dsDNA Positive Control in 1.0 ml DNA Diluent.
  - c) Dilute 10µl of anti-dsDNA Calibrator in 1.0 ml DNA Diluent.
  - d) Dilute 10µl of anti-dsDNA Negative Control in 1.0 ml DNA Diluent.
  - e) Discard excess working solutions after use.

## **Anti-dsDNA TEST PROCEDURE**

(See Procedure Notes and Pre-Test Setup Instructions)

- 1) Apply to assigned wells:

Controls - 100µl (diluted 1:100 with DNA Diluent).  
Patient samples - 100µl of patient serum (diluted 1:100 with DNA Diluent).  
Blanking Control - 100µl DNA Diluent.

- 2) Incubate wells: Shake plate gently, then incubate for 30 minutes at room temperature. (Do not incubate for more than 40 minutes.)
- 3) Discard incubated samples: After 30-minute incubation, discard samples by inverting plate and rapidly flicking the liquid away from the plate.
- 4) Wash wells 5x: Gently fill with 200µl Wash Solution and discard. Remove all liquid before proceeding.
- 5) Apply HRP reagent: Add 100µl HRP Anti Human IgG to all wells. (Avoid contamination of the HRP.) Discard excess transferred HRP after use.
- 6) Incubate wells: Shake plate gently, then incubate for 30 minutes at room temperature. (Do not incubate HRP for more than 40 minutes.)
- 7) Discard incubated HRP: After 30-minute incubation, discard HRP by inverting plate and rapidly flicking the liquid away from the plate.
- 8) Wash wells 5x: Gently fill with 250µl Wash Solution and discard. Remove all liquid before proceeding.
- 9) Develop color: Add 100µl of TMB color reagent to each well. Discard excess transferred TMB after use.
- 10) Incubate wells: Shake or tap plate gently to disperse color. Incubate for 30 minutes at room temperature.
- 11) Stop color development: After 30-minute color development, add 100µl of Stop Buffer to each well to stop the color development.
- 12) Read results: Read wells within 30 minutes with an EIA reader set to 450 nm. First, zero the reader on the DNA Diluent blanking control well, then read the color of the control and patient wells. The anti-dsDNA Positive Control well should show yellow color. The anti-dsDNA Calibrator well should show yellow color. The anti-dsDNA Negative Control well should show little color. The DNA Diluent blanking control well should show little color or be clear.

## QUALITY CONTROL

In order for a test to be valid, all of the following criteria must be met:

- 1) An anti-dsDNA Positive Control, anti-dsDNA Calibrator, anti-dsDNA Negative Control, and DNA Diluent must be included with each test run.
  - 2) The absorbance values for each control must be within the specified range printed on the quality control card included with each kit lot number. (Do not use quality control cards from other kit lot numbers.)
  - 3) The DNA Diluent OD must be  $\leq 0.200$  when zeroed against air.
- If any of these criteria are not met, the results are invalid and the test should be repeated.

## RESULTS

Microtiter strip wells must be read with an EIA reader set to 450nm. Results should be read after adding the Stop Buffer (Step 11) and reported as follows:

Positive: A positive response is indicated by a yellow color; the calculated IUs are greater than 25.

Negative: A negative response is indicated by a colorless, or less intense yellow color. The calculated IUs are less than 25.

### Calculation of Results:

Determine the International Units (IUs) based on the WHO Wo-80 standard for each patient specimen (or control) using the following formula:

$$\frac{\text{IU of Calibrator}}{\text{OD of Calibrator}} \times \text{OD of Test Sample} = \text{IU of Test Sample}$$

### Interpretation of Results:

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

IU's	Interpretation of Results
Less than 25	Negative
25 to 30	Borderline Positive

30 to 60	Low Positive
60 to 200	Positive
Greater than 200	Strong Positive

Retest borderline samples before reporting results. If repeated results are still borderline, the test sample has no significant antibodies.

**LIMITATIONS**

As with other diagnostic tests, the results are to be used as an aid in diagnosis. A positive result suggests certain diseases and should be confirmed by clinical findings.

**EXPECTED VALUES**

The negative range was determined from serum samples obtained from 78 normal blood donors, which were assayed by the EIA anti-dsDNA test. The Average IU = 2.5 and Standard Deviation = 2.4. All 78 normal samples were negative for dsDNA antibodies.

The positive range was established using data obtained from 67 control sera and patient sera containing dsDNA antibodies. IU ranged from a low of 33 to a high of 425 (14). Anti-dsDNA antibodies are not expected in a normal population (10). The following are the frequency of anti-dsDNA antibodies found in autoimmune diseases (15): SLE - 40%; MCTD - 0%; Sjogren's Syndrome - 0%; Drug Induced Lupus - 0%; Progressive Systemic Sclerosis - 0%; Dermatomyositis/Polymyositis - 0%.

**PERFORMANCE CHARACTERISTICS**

To demonstrate the specificity of the EIA Anti-dsDNA assay, a number of ANA specific control sera containing high levels of antibodies to dsDNA, Sm, RNP, SS-A/Ro, SS-B/La, Jo-1, Scl-70, Centromere, and Histones were tested using the Anti-dsDNA test. All anti-dsDNA sera gave positive results. All sera containing other specificities were negative for anti-dsDNA (14).

A side by side comparison with another commercially available ELISA Anti-dsDNA assay was done on 204 serum samples obtained from a population containing both positive and negative samples. The overall agreement was 96.1% when compared to the reference method. Disregarding the borderline samples, the relative sensitivity and specificity are 100% and 99.2%, respectively.

Intra-assay precision was determined by testing a strong positive anti-dsDNA control and a weak positive anti-dsDNA control with a replication of 18. The CVs were 9.8% and 8.8%. Inter-assay precision was determined by testing a strong positive anti-dsDNA control and a weak positive anti-dsDNA control in a total of 12 assays. The CVs were 10.7% and 11.7% (14).

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