Anti-dsDNA ELISA

For in vitro diagnostic use.
Catalog No. 2553

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).

SIGNIFICANCE AND BACKGROUND
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 OF THE ELISA ASSAY
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.

KIT COMPONENTS

- **HRP-CONJUGATE REAGENT** 1vial
  15 ml per vial.
  Horseradish peroxidase-conjugated goat anti-human IgG.
  Ready to use as supplied.
  Once open, stable for one month at 2-8°C.
- **WASH BUFFER [16.7X]** 1bottle
  DILUTE BEFORE USE
  60 ml per bottle containing 16.7X concentration of PBS and Tween-20 solution.
  Diluted solution will have a pH of 7.3±0.2.
  Once diluted, stable for one week at room temperature or for two weeks when stored at 2-8°C.
- **TMB** 1bottle
  TMB solution (3',5'-Tetramethyl benzidine) in dilute H2O2 buffer.
  Ready to use as supplied.
  Once open, stable for one month at 2-8°C.
- **STOP SOLUTION** 1bottle
  15 ml per bottle.
  H2SO4 (1.5%) and HCl (1.5%), pH <3.0.
  Ready to use as supplied.
- **SAMPLE DILUENT** 4 bottles
  30ml bottle
  PBS, (pH 7.3±0.2), with <0.1% sodium azide as a preservative.
  Ready to use. Note: Shake well Before Use.
- **PACKAGE INSERTS** 1copy

- **MICROWELL PLATE** 1plate
  dsDNA antigen-coated microwell strips fixed on a strip holder.
  The plate is sealed in aluminum pouch with desiccant.
  8×12/12×8-well strips per plate.
- **NEGATIVE CONTROL** 1vial
  0.45 ml per vial.
  Human Serum. Preservative added.
  Ready to use as supplied.
  Once open, stable for one month at 2-8°C.
- **POSITIVE CONTROL** 1vial
  0.45 ml per vial.
  Human Serum. Preservatives added.
  Ready to use as supplied.
  Once open, stable for one month at 2-8°C.
- **CALIBRATOR** 1vial
  0.45 ml per vial.
  Human Serum. Preservative and buffer added.
  Ready to use as supplied.
  Once open, stable for one month at 2-8°C.
ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

- Freshly distilled or deionized water.
- Disposable gloves and timer.
- Appropriate waste containers for potentially contaminated materials.
- Disposable V-shaped troughs.
- Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
- Absorbent tissue or clean towel.
- Dry incubator or water bath, 37±0.5°C.
- Microshaker for dissolving and mixing conjugate with samples.
- Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
- Microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. **Sample Collection:** Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

2. **Transportation and Storage:** Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C; do not freeze. To assure maximum performance of this Anti-dsDNA ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

This kit is intended FOR IN VITRO USE ONLY [IVD] FOR PROFESSIONAL USE ONLY

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.

2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.

3. CAUTION - CRITICAL STEP: Allow the reagents and samples to stabilize at room temperature(18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.

4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.

5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.

6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.

7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.

8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.

9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.

10. The use of automatic pipettes is recommended.

11. Ensure that the incubation temperature is 37°C inside the incubator.

12. When adding samples, avoid touching the well’s bottom with the pipette tip.

13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.

14. All specimens from human origin should be considered as potentially infectious.

15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, Smoke, or apply cosmetics in the assay laboratory.

16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.

17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1 hour at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps for disposal.

18. The Stop solution (H2SO4, HCl ) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.

20. Transfer to a graduated test tube 1mL of Conjugate for each strip to be run. Discard excess transferred Conjugate.

21. Transfer to a graduated test tube 1mL of TMB for each strip to be run. Discard excess transferred TMB.

22. Materials Safety Data Sheet (MSDS) available upon request.

23. If using fully automated micropate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

TEST PROCEDURE

Special Instructions for Washing

1. A good washing procedure is essential to obtain correct and precise analytical data. Because the DNA Wash Concentrate contains salts, crystals, may form in the concentrated solution. For proper preparation of the Wash Solution, complete the following steps.

   1. Empty contents of DNA Wash Concentrate bottle, including any crystals into a 1L bottle.

   2. If any crystals remain in the DNA Wash Concentrate bottle, remove them by adding some deionized water to the bottle; mix and pour all contents into the 1L bottle.

   3. Add deionized water to the 1L bottle to bring the final volume to 1L.
4. Place a stir bar in the 1L bottle and place on a stir plate. Stir the diluted Wash Solution for a few minutes until all crystals are dissolved. If no stir plate is available, cover the top of the Wash Solution and gently invert back and forth until the crystals are dissolved. Avoid excessive bubbles.

2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).

3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.

4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.

5. In case of manual washing, we suggest to perform at least 5 cycles, dispensing 350-400µl/well and aspirating the liquid for 5 seconds. If poor results (high background) are observed, increase the washing cycles or soaking time per well.

6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution (final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.

**Preparation of Reagents:**

Dilute 10µL of patient's sera in 1.0mL of DNA Sample Diluent.
1. Dilute 10µL of anti-dsDNA positive control in 1.0mL of DNA sample diluent.
2. Dilute 10µL of anti-dsDNA negative control in 1.0mL of DNA sample diluent.
3. Dilute 10µL of anti-dsDNA Calibrator in 1.0mL of sample diluent.
4. Discard excess solutions after use. Always use fresh sample dilutions.

**Assay Procedure:**

1. Remove the individual kit components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the total number of microwells needed. Allow six control/calibrator determinations (one blank, one control, three calibrators and one positive control) per run. A reagent blank should be run on each assay. Check software and reader requirements for the correct control/calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8° C.
3. To individual wells, add 100ul of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
4. Add 100ul of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configurations.
5. Incubate the plate in room temperature (20-25°C) for 30 minutes. (Do not incubate diluted sera for more than 40 min.)
6. Wash the microwell strips 5X.
   **A. Manual Wash Procedure:**
   a. Vigorously shake out the liquid from the wells.
   b. Fill each microwell with 200µL of Wash Buffer. Make sure no air bubbles are trapped in the wells.
   c. Repeat steps a. and b. for a total of 5 washes.
   d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.
   **B. Automated Wash Procedure:**
   If using an automated microwell wash system, set the dispensing volume to 300-350 µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
7. Add 100ul of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
8. Incubate the plate at room temperature (20-25°C) for 30 minutes. (Do not incubate for more than 40 min.) After 30 min, discard Conjugate by inverting plate and rapidly flicking the liquid away from the plate.
9. Wash the microcells by following the procedure as described in step 6.
10. Add 100ul of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
11. Incubate the plate at room temperature (20-25°C) for 30 minutes.
12. Stop the reaction by adding 100µL of stop solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added.
13. Set the microwell reader to read at a wavelength of 450 nm and measure the optical density (OD) of each against the reagent blank. The plate should be read within 30 minutes after the addition of the stop Solution.

**INTERPRETATION OF RESULTS**

**A. Calculations:**

Determination of International Units (IU) based on the WHO Wo/80 standard for each patient specimen (or control) using the following formula:

\[
\text{IU of Calibrator} \times \text{OD of Test Sample} = \text{IU of Test Sample OD of Calibrator}
\]

The Anti-dsDNA Calibrator (100IU) is equal to 0.5X the Wo/80 reference material (200 IU).

**B. 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 Negative Control, and DNA Sample Diluent Blank must be included with each test run.
2. The values for each control must be within the specified range printed on the quality control card included with each kit lot number.
3. The DNA Sample Diluent OD must be < 0.200 when zeroed against air.

If any of these criteria are not met, the results are considered invalid and the test should be repeated.

**C. Results:**

Microtiter strips wells must be read with an EIA reader set to 450nm. Results should be read after adding the Stop Solution (Step 12) and reported as follows:

- **Positive:** A positive response is indicated by a yellow color; the calculated IUs are > 25.
- **Negative:** A negative response is indicated by a colorless, or less intense yellow color; the calculated IUs are < 25.

**LIMITATIONS**

1. The Anti-dsDNA ELISA test is a diagnostic aid and by itself is not diagnostic. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. Positive Anti-dsDNA may be found in apparently healthy people. It is therefore imperative that the results be interpreted in light of the patient's clinical picture by a medical authority.
3. Many commonly prescribed drugs may induce Anti-dsDNA.
EXPECTED VALUES

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.

<table>
<thead>
<tr>
<th>IU Range</th>
<th>Interpretation of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>Negative</td>
</tr>
<tr>
<td>25 to 30</td>
<td>Borderline Positive</td>
</tr>
<tr>
<td>30 to 60</td>
<td>Low Positive</td>
</tr>
<tr>
<td>60 to 200</td>
<td>Positive</td>
</tr>
<tr>
<td>&gt;200</td>
<td>Strong Positive</td>
</tr>
</tbody>
</table>

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

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 the Standard Deviation = 2.4. All the 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. 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

Specificity
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).

Accuracy
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.

Precision
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).

REFERENCES

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