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IVD	 See external label	 2°C-8°C	 Σ=96 tests	REF Cat # 2553Z
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Anti-dsDNA ELISA

Cat # 2553Z

Test	Anti -ds DNA ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antigen Coated Plate
Detection Range	Qualitative: Positive and Negative Control
Sample	100µl serum
Specificity	99.2%
Sensitivity	100%
Total Time	~90min
Shelf Life	12 months

INTENDED USE

The Diagnostic Automation Inc. Autoimmune EIA 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).

For In Vitro Diagnostic Use.

SUMMARY AND EXPLANATION OF THE TEST

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 50-70% 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 PROCEDURE

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 micro-wells 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 450 nm.

KIT COMPONENTS

The EIA Anti-dsDNA Test Kit contains supplies for 96 or 576 tests:

Component	REF	Description	96 Test Kit REF 96DS	576 Test Kit 576DS
Anti-dsDNA Microplate MPLT	2553Z-DA	DsDNA antigen-coated wells, sealed in a resealable foil pouch with desiccant.	96 Wells	6 x 96 wells
DNA Wash Concentrate (16.7X) WSH CONC	2553Z-DA	Phosphate buffer, pH 7.3+/-0.2 (when diluted to working solution), with Tween-20 as a detergent. Use to wash wells.	1 x 60 mL	6 x 60 mL
DNA Sample Diluent SAMP DIL	2553Z-DA	Phosphate buffer, pH 7.3+/-0.2 with <0.1% sodium azide as a preservative. Use to dilute patient samples, calibrator, and positive and negative controls. Use for Blanking Control.	4 x 30 mL	24 x 30 mL
Conjugate CONJ	2553Z-DA	Goat anti-human IgG horseradish peroxidase in buffer, pH 6.2-6.7.	1 x 15 mL	6 x 15 mL
Anti-dsDNA Positive Control CONTROL +	2553Z-DA	Human serum positive for anti-dsDNA antibodies.	1 x 0.45 mL	6 x 0.45 mL
Anti-dsDNA Calibrator CAL	2553Z-DA	Human serum with anti-dsDNA antibodies. Use to calculate sample's International Units (IUs).	1 x 0.45 mL	6 x 0.45 mL
Anti-dsDNA Negative Control CONTROL -	2553Z-DA	Human serum negative for anti-DNA antibodies.	1 x 0.45 mL	6 x 0.45 mL
Substrate SUBS	2553Z-DA	Tetramethylbenzidine (TMB) in dilute hydrogen peroxide buffer.	1 x 15 mL	6 x 15 mL
Stop Solution STOP	2553Z-DA	Contains sulfuric acid (1.5%), pH <3.0. Use to stop color development.	1 x 15 mL	6 x 15 mL

ADDITIONAL REQUIRED ITEMS

EIA Reader (set to 450 nm)
Micropipettors (10 μ L & 100 μ L)
8-channel Repeating Pipettor (for washing)
Deionized (DI) Water
Pipettes (1 mL & 10 mL)
Graduated Cylinder (\geq 120 mL)
1 Liter Container (for DNA Wash Solution)
Test Tubes (4 mL & 15 mL)
Countdown Timer

RECOMMENDED ITEMS

Automatic Washer
100 μ L 8-Channel Micropipettor (for reagent delivery)
1 mL Mini-Tubes (for sample dilutions)

PRECAUTIONS/WARNINGS

1. The Stop Solution 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.
2. Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
3. Do not smoke, eat, or drink in areas where patient samples and kit reagents are handled.
4. Do not pipette by mouth.
5. Wear personal protective equipment while handling all reagents and samples and while operating the washer and reader.
6. Dispose of all wastes in accordance with applicable national and/or local regulations.
7. Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
8. Waste material containing patient samples or biological products should be considered biohazardous when disposing or treating.
9. Chemical reagents should be handled in accordance with Good Laboratory Practices.
10. Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
11. Do not use kit beyond its expiration date. The date is printed on kit boxes.
12. This product uses human serum in the manufacture of the Calibrator and Controls. Each unit was tested by FDA accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV) Hepatitis C (HCV) and syphilis. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human source material should be considered potentially infectious for all infectious agents; therefore, handle the Calibrators and Controls with the same precautions used with patient specimens.
13. Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
14. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
15. Approximately 30 minutes before beginning the assay, remove the kit from the refrigeration (2–8 $^{\circ}$ C) and allow the kit components to come to room temperature

(18–27 °C). Mix reagents thoroughly by gently swirling the container several times before use. Return the assay materials to 2–8 °C after use.

16. Do not interchange reagents between kit lots.

PROCEDURE NOTES

1. All materials must be at room temperature (18-27 °C) before beginning the assay.
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 Sample Diluent. Prepare all dilutions before starting test. Always use fresh sample dilutions.
8. Always run an anti-dsDNA Positive Control, an anti-dsDNA Calibrator, and an anti-DNA Negative Control. Always blank against DNA Sample 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, 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 Conjugate to incubate in the strip wells for more than 40 minutes. For best results, use 1 mL minitubes to prepare sample dilutions. Transfer all solutions into wells with an 8-channel Micropipettor.
11. After each incubation, thoroughly wash the microtiter wells with ~200 µL DNA 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 Conjugate for each strip to be run. Discard excess transferred Conjugate. Transfer to a graduated test tube 1mL of Substrate for each strip to be run. Discard excess transferred Substrate.

SPECIMEN COLLECTION AND HANDLING

1. Specimen Type Serum.
2. Specimen Collection Precaution
3. Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
4. Specimen Additives, Preservatives Collect blood aseptically in untreated tubes.
5. Specimen Pretreatment/Storage
6. Allow blood to clot. Separate serum immediately. Avoid use of lipemic, hemolyzed or contaminated sera. Store sera at 2-8 °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.

PREPARATION 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.

- Collect all reagents, samples and dilutions necessary before starting assay.
- Assign and record wells for controls and samples.

Anti-dsDNA Microplate

1. Ready to use.
2. After opening the foil pouch, the wells are stable for 30 days if immediately returned to resealed foil pouch with desiccant.

EIA Frame DNA Wash Solution

- Retain for future use.

DNA Wash Solution

Because the DNA Wash Concentrate contains salt, 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 1 L 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 1 L bottle.
3. Add deionized water to the 1 L bottle to bring the final volume of the solution to 1 liter.
4. Place a stir bar in the 1 L 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.
5. Diluted DNA Wash Solution is stable for 14 days at 2-8 °C.
6. Retain for future use.

DNA Sample Diluent

1. Ready to use.
2. Allow DNA Sample Diluent to reach room temperature before use.
3. Mix thoroughly.
4. Avoid unnecessary contamination.

Conjugate, Substrate and Stop Solution

- Ready to use.

1:100 Working Solutions

Prepare as Follows:

1. Dilute 10 µL of patient's sera in 1.0 mL of DNA Sample Diluent.
2. Dilute 10 µL of anti-dsDNA Positive Control in 1.0 mL of DNA Sample Diluent.
3. Dilute 10 µL of anti-dsDNA Calibrator in 1.0 mL of DNA Sample Diluent.
4. Dilute 10 µL of anti-DNA Negative Control in 1.0 mL of DNA Sample Diluent.
5. Discard excess working solutions after use.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Do not use any reagents that show signs of leakage.

PROCEDURE

Assay Steps

1. Apply diluted samples and controls to wells:
 - Controls - Apply 100 μ L of diluted controls (1:100 in DNA Sample Diluent) to assigned wells. Add 100 μ L of DNA Sample Diluent as a blank control.
 - Patient samples - Apply 100 μ L of diluted patient serum (1:100 in DNA Sample Diluent) to assigned wells.
2. Incubate wells: Shake plate gently, then incubate for 30 minutes at room temperature (18-27 °C). (Do not incubate diluted sera in wells 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 200 μ L DNA Wash Solution and discard. Remove all liquid before proceeding.
5. Apply Conjugate: Add 100 μ L Conjugate to all wells. (Avoid contamination of the Conjugate.) Discard excess transferred Conjugate after use.
6. Incubate wells: Shake plate gently, then incubate for 30 minutes at room temperature (18-27 °C). (Do not incubate Conjugate for more than 40 minutes.)
7. Discard incubated Conjugate: After 30 flicking the liquid away from the plate.
8. Wash wells 5x: Gently fill with 200 μ L DNA Wash Solution and discard. Remove all liquid before proceeding.
9. Develop color: Add 100 μ L of Substrate to each well. Discard excess transferred Substrate after use.
10. Incubate wells: Shake or tap plate gently to disperse color. Incubate for 30 minutes at room temperature (18-27 °C).
11. Stop color development: After 30 minute color development, add 100 μ L of Stop Solution to each well to stop the color development. Read results: Read wells within 30 minutes with an EIA reader set to 450 nm. First, zero the reader on the DNA Sample 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-DNA Negative Control well should show little color. The DNA Sample Diluent blanking control well should show little color or no color.

QC Requirements

An anti-dsDNA Positive Control, anti-dsDNA Calibrator, anti-dsDNA Negative Control, and DNA Sample Diluent Blank must be included with each test run.

GUIDELINES FOR THE INTERPRETATION OF RESULTS

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-DNA 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 invalid and the test should be repeated.

Results:

Microtiter strip wells must be read with an EIA reader set to 450 nm. Results should be read after adding the Stop Solution (Step 11) 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 .

Calculation of Results:

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

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

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

LIMITATIONS OF THE PROCEDURE

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

EXPECTED VALUE RANGE

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.

IUs	Interpretation of Results
< 25	Negative
25 to 30	Borderline Positive
30 to 60	Low Positive
60 to 200	Positive
> 200	Strong Positive

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 Standard Deviation = 2.4. All 78 normal sam- ples 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:14 SLE - 40%; MCTD -

0%; Sjögren'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.

Accuracy

A side-by-side comparison with another commercially available ELISA Anti-dsDNA assay was performed 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% respectively.

Inter-assay precision 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 10.0% and 8.8% respectively.

PRODUCT SAFETY INFORMATION

No safety information to report for this product.

REFERENCES

1. White, R. H.; Robbins, D. L. Clinical Significance and Interpretation of Antinuclear Antibodies. *West. J. Med.* 1987, 147, 210-213.
2. Hardin, J. A. The Lupus Autoantigens and The Pathogenesis of Systemic Lupus Erythematosus. *Arthritis Rheum.* 1986, 29 (4), 457-460.
3. Condemi, J. J. The Autoimmune Diseases. *JAMA.* 1987, 258 (20), 2920-2929.
4. Tan, E. M.; Cohen, A. S.; Fries, J. F.; Masi, A. T.; McShane, D. J.; Rothfield, N. F.; Schaller, J. G.; Talal, N.; Winchester, R. J. The 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus. *Arthritis Rheum.* 1982, 25 (11), 1271-1277.
5. Tan, E. M.; Schur, P. H.; Carr, R. I.; Kunkel, H. G. Deoxyribonucleic Acid (DNA) and Antibodies to DNA in the Serum of Patients with Systemic Lupus Erythematosus. *J. Clin. Invest.* 1966, 45 (11), 1732-1740.
6. Koffler, D. Immunopathogenesis of Systemic Lupus Erythematosus. *Annu. Rev. Med.* 1974, 25, 149-164.
7. Emlen, W.; Pisetsky, D. S.; Taylor, R. P. Antibodies to DNA: A Perspective. *Arthritis Rheum.* 1986, 29 (12), 1417-1426.
8. Pincus, T.; Schur, P. H.; Rose, J. A.; Decker, J. L.; Talal, N. Measurement of Serum DNA-Binding Activity in Systemic Lupus Erythematosus. *N. Engl. J. Med.* 1969, 281 (13), 701-705.
9. Minitzer, M. F.; Stollar, B. D.; Agnello, V. Reassessment of the Clinical Significance of Native DNA Antibodies in Systemic Lupus Erythematosus. *Arthritis Rheum.* 1979, 22 (9), 959-968.
10. Emlen, W.; Jarusiripipat, P.; Burdick G. A New ELISA for the Detection of Double-Stranded DNA

Antibodies. J. Immunol. Methods 1990, 132, 91-101.

11. Notman, D. D.; Kurata, N.; Tan E. M. Profiles of Antinuclear Antibodies in Systemic Rheumatic Diseases. Ann. Intern. Med. 1975, 83, 464-469.
12. Locker, J. D.; Medof, M. E.; Bennett, R. M.; Sukhupnyaraksa, S. Characterization of DNA Used to Assay Sera for Anti-DNA Antibodies; Determination of the Specificities of Anti-DNA Antibodies in SLE and Non-SLE Rheumatic Disease States. J. Immunol. 1977, 118 (2), 694-701.
13. Smeenk, R. J. T.; Brinkman, K.; Brink, H. G.; Westgeest, A. A. A. Reaction Patterns of Monoclonal Antibodies to DNA. J. Immunol. 1988, 140 (11), 3786-3792.
14. Tan, E. M. Antinuclear Antibodies: Diagnostic Markers for Autoimmune Diseases and Probes for Cell Biology. Adv. Immunol. 1989, 44, 93-151.

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