Human dsDNA IgG ELISA Kit

Cat.No:DEIA1696

Lot. No. (See product label)

PRODUCT INFOMATION

Storage	1. Store the unopened kit at 2-8 °C. 2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator remains blue. 3. Conjugate: Store at 2-8 °C. DO NOT FREEZE. 4. Calibrator, Positive Control and Negative Control: Store at 2-8 °C. 5. TMB: Store at 2-8 °C. 6. Wash buffer concentrate (10X): Store at 2-25 °C. Diluted wash buffer (1X) is stable at room temperature (20 to 25 °C) for up to 7 days or for 30 days between 2 and 8 °C. 7. Sample Diluent store between 2 and 8 °C. 8. Stop Solution: Store between 2-25 °C.
Pkg#Size	96T
Intended use	The dsDNA ELISA test system is a semi-quantitative immunoassay for the detection of IgG antibodies to double stranded DNA (dsDNA) in human sera. When performed according to these instructions, the results of this autoantibody ELISA may aid in the diagnosis and treatment of autoimmune connective tissue disorders.
Principle Of The Test	The dsDNA ELISA test system is designed to detect IgG class antibodies in human sera to purified double stranded DNA. The test procedure involves three incubation steps: 1. Test sera (properly diluted) are incubated in a series of microwells coated with purified dsDNA. If present in patient sera, specific antibodies will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components. 2. Peroxidase conjugated goat anti-human IgG (chain specific) is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate. 3. The microwells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the test sample.
Reagents And Materials Provided	Each kit contains the following components in sufficient quantities to performa the number of tests indicatee on packaging label. 1. Plate. 96 wells configured in twelve 1x8 well strips coated with inactivated antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant. 2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG (y chaing specific). Ready to use. One, 15 mL vial with a white cap. Preservative added. 3. Positive Control (Human Serum). One, 0.35 mL vial with a red cap. Preservative added. 4. Calibrator (Human Serum). One, 0.5 mL vial with a blue cap. Preservative added. 5. Negative Control (Human Serum). One, 0.35 mL vial with a green cap. Preservative added. 6. Sample diluent. One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-bufferedsaline, (pH 7.2 ±/- 0.2). Green solution ready to use. Note: Shake Well Before Use. Preservative added. 7. TMB: One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO 8. Stop solution: One 15 mL bottle (red cap) containing 1M H2SO4, 0.7M HCI. Ready to use. 9. Wash buffer concentrate (10X): dilute 1 part concentrate ± 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). Contains preservative. NOTE: 1X solution will have a pH of 7.2 ±/- 0.2.

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Materials Required But Not Supplied	1. ELISA microwell reader capable of reading at a wavelength of 450nm. 2. Pipettes capable of accurately delivering 10 and 200µL. 3. Multichannel pipette capable of accurately delivering (50-200ul). 4. Reagent reservoirs for multichannel pipettes. 5. Wash bottle or microwell washing system. 6. Distilled or deionized water. 7. One liter graduated cylinder. 8. Serological pipettes. 9. Disposable pipette tips. 10. Paper towels 11. Laboratory timer to monitor incubation steps. 12. Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)
Assay Steps	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 samples and controls to be tested. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should br un on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 and 8°C. 3. Prepare a 1:21 dilution e.g.: 10 uL of serum ± 200ul of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. 4. To individual wells, add 100 ul of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample. 5. Add 100 ul of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration. 6. Incubate the plate at room temperature (20-25°C) for 25 ±/- 5 minutes. 7. Wash the microwell strips, 5X. A. Manual Wash procedure: a. Vigorously shake out the liquid from the wells. b. Fill each well with 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 wash system, set the dispensing volume to 300-350µL/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. 8. Add 100µL of the TMB substrate solution to each well at the same rate and in the same order as the specimens were added. 9. Incubate the plate at room temperature (20-25°C) for 25 ±/- 5 minutes. 10. Wash the microwells by following the procedure as described in step 7. 11. Add 100 ul of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added. 12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes. 13. Stop the reaction by adding 50 ul of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed. 14. Set the microplate reader wavelength at 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

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Quality Control	1. Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay. 2. Calculate the mean of three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells. 3. The mean OD value for the Calibrator and the OD values fro the Positive and Negative Controls should fall with the following ranges: Negative Control <> Calibrator >0.300 Positive Control >0.500 a. The OD of the Negative Control divided by the mean OD of the Calibrator should be >0.9. b. The OD of the Positive Control divided by the mean OD of the Calibrator should be >1.25. c. If the above conditions are not met the test should be considered invalid and should be repeated. 4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off. 5. Additional controls may be tested according to guidelines or requirements of local state, and or federal regulations or accrediting organizations.

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Precautions	1. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws. 2. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered POTENTIALLY BIOHAZARDOUS MATERIALS and handled accordingly. 3. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institute of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (20). 4. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach room temperature (20-25°C) before starting the assay. Return unused reagents to refrigerated temperature immediately after use. 5. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations. 6. The human serum controls, Sample Diluent, Conjugate, and Wash Buffer concentrate contain a preservative (sodium azide, 0.1% (w/v) react with laboratory plumbing which may cause explosion on hammering. 7. The Stop Solution is TOXIC. Causes burn. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately. 8. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin. 9. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin. 10. Wipe bottom of plate free of residual liquid and/or fingerprints, which can alter optical density (OD) readings. 11. Dilution or adulteration of these reagents may generate erroneous results. 12. Reagents from other sources or manufacturers should not be used. 13. TMB Solution should be colorless, very pale yellow, very pale green or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test procedure, Substrate Incubation section to determine the amount of TMB to be used. 14. Never pipette by mouth. Avoid contact or reagents and patient specimens with skin and mucous membranes. 15. Avoid microbial contamination of reagents. Incorrect results may occur. 16. Cross contamination of reagents and/or samples could cause erroneous results. 17. Reusable glassware must be washed out and thoroughly rinsed free of all detergents. 18. Avoid splashing or generation of aerosols. 19. Do not expose reagents to strong light during storage or incubation. 20. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation. 21. Wash solution should be collected in a disposal basin. Treat the waste solution with 10 household bleach (0.% sodium hypochlorite). Avoid exposure to reagents to bleach fumes. 22. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution. 23. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink. 24. Do not allow the conjugate to come in contact with containers or instruments, which may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity. 25. Do not expose any of the reactive reagents to bleach-containing solutions, or to any strong odors from bleachcontaining solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.
Specimen Collection And Handling	1. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious. 2. Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera. 3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

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Reference Values	The expected value for a normal patient is a negative result. The number of reactives, and the degree of reactivity is dependent upon parameters such as population type being tested, treatment, etc. Each laboratory should establish their own expected values based upon the specimens typically being tested. With respect to disease-state and percent reactivity, Table I in the SIGNIFICANCE AND BACKGROUND section of this package insert shows the relative frequency of autoantibody activity for various rheumatic disorders.
General Description	Deoxyribonucleic acid is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms (with the exception of RNA viruses). The DNA segments carrying this genetic information are called genes. Likewise, other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information. Along with RNA and proteins, DNA is one of the three major macromolecules that are essential for all known forms of life. DNA consists of two long polymers of simple units called nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds. These two strands run in opposite directions to each other and are therefore anti-parallel. Attached to each sugar is one of four types of molecules called nucleobases (informally, bases). It is the sequence of these four nucleobases along the backbone that encodes information. This information is read using the genetic code, which specifies the sequence of the amino acids within proteins. The code is read by copying stretches of DNA into the related nucleic acid RNA in a process called transcription. Within cells DNA is organized into long structures called chromosomes. During cell division these chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes. Eukaryotic organisms (animals, plants, fungi, and protists) store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm. Within the chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.
Interpretation of Results	A. Calculations: 1. Correction Factor A cutoff OD value for the positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cut-off value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the data label located in the kit box. 2. Cutoff OD Value To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above. 3. Index Values or OD Ratios Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from Step 2. B. Interpretations: Index Values or OD ratios are interpreted as follows: Negative Specimens <> equivocal Specimens 0.91 to 1.09 Positive Specimens >1.10
Reproducibility	To assess the intra-assay and inter-assay variability of the test procedure, a strong positive, a low positive, and a negative sample was tested eleven times on each of three days. The mean unit value, the standard deviation, and the percent CV were calculated for each sample.
Limitations	1. A diagnosis should not be made solely on the basis of the dsDNA ELISA test results. 2. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.

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