Human Cardiolipin IgA ELISA Kit

Cat.No:DEIA1710

Lot. No. (See product label)

PRODUCT INFOMATION

Storage	1. Store the unopened kit at 2° and 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 strip on the desiccant pouch remains blue. 3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE. 4. Calibrator, Positive Control and Negative Control: Store between 2° and 8°C. 5. TMB: Store between 2° and 8°C. 6. Wash Buffer concentrate (10X): Store between 2 and 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 at 2° and 25°C.
specificity	To investigate the potential for positive reactions due to cross reactive antibodies, fourteen specimens which were reactive for various auto-antibodies were tested on the anti-Cardiolipin test system. Ten of the fourteen (10/14, 71.4%) were negative for anti-Cardiolipin IgA activity, while four of the fourteen (4/14, 28.6%) were positive. The results of this study indicate that the potential for a high degree of cross reactivity with such autoantibodies is not likely.
Pkg#Size	96T
Intended use	The Cardiolipin Antibody Test System is an enzyme-linked immunosorbent assay (ELISA) designed for the semiquantitative measurement of circulating IgA autoantibodies to cardiolipin.
Principle Of The Test	The Cardiolipin IgA ELISA test system is designed to detect IgA class antibodies to Cardiolipin in human sera. Wells of plastic microwell strips are sensitized by passive absorption with Cardiolipin antigen. The test procedure involves three incubation steps: 1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components. 2. Peroxidase conjugated goat anti-human IgA is added to the wells and the plate is incubated. The conjugate will react with antibody immobilized on the solid phase in step 1. The wells are washed to remove un-reacted 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 original test sample.
Reagents And Materials Provided	1. Plate. 96 wells configured in twelve 1x8-well strips coated with Cardiolipin antigen from bovine heart. The strips are packaged in a strip holder and sealed in an envelope with desiccant. 2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG. (γ chain 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 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 phosphae-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',,'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO 8. Stop Solution: One 15 mL bottle (red cap) containing 1M H2S04, 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 (clear 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 to 200µl. 3. Mulltichannel pipette capable of accurately delivering (50-200µl) 4. Reagent reservoirs for multichannel pipettes. 5. Wash bottle or microwell washing system. 6. Distilled or deionized water. 7. One liter graduated cylinder. 8. Seroligical 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 components from storage and allow them to warm to room temperature (20-25 °C) 2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative 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 Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and returned to storage between 2° and 8 °C. 3. Prepare a 1:21 dilution (e.g.: 10 µL of serum ± 200 µL 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 100µl of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct 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 Prcedure: 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 five 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 (10% household bleach) at the end of the days run. B. Automated Wash procedure: If using an automated microwell wash system, set the dispensing volume to 300-350µL/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 conjugate 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 previously described in step 7. 11. Add 100µL 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 50ul 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 microwell reader to read at a wavelength of 450 nm 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 positive calibrator should be run in triplicate. A positive and negative control, and reagent blank must also be included in each assay.
2. Calculate the mean of the three positive calibrator determinations. If any of the three positive calibrator values differ by more than 15% from the mean, discard that value and calculate the mean of the remaining two values.
3. The mean OD value for the positive calibrator and the OD values for the positive and negative controls should fall within the following ranges:
OD Range
Negative Control <>
Positive Calibrator >0.300
Positive Control >0.500
a. The OD of the negative control divided by the mean OD of the positive calibrator should be <>
b. The OD of the positive control divided by the mean OD of the positive calibrator should be >1.25.
c. If the control values are not within the above ranges, the test should be considered invalid and the test 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. The positive and negative controls must meet the following additional criteria:
a. The negative control must be
b. The positive control must be >25 GPL.

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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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Calculation	1. Positive Calibrator Based upon testing of normal and disease-state specimens, a maximum normal unit (AAU) value has been determined by the manufacturer and correlated to the positive calibrator. The calibrator will allow you to determine the unit value of test samples, and to correct for slight day-to-day variations in test results. The calibrator unit value is determined for each lot of kit components and is printed on the Component List. 2. Conversion of Optical Density to U/mL. The conversion of OD to unit value (APL) can be represented by the following equation: Test Specimen APL = (A x B)/C Where: APL = Unknown unit value to be determined A = OD of test specimen in question. B = Unit value of calibrator (APL). C = The mean OD of calibrator. Example: Test specimen OD for PR-3 = 0.946 Calibrator OD for Cardiolipin = 0.435 Calibrator unit value for Cardiolipin = 155 APL Test Specimen APL = (0.946 x 155)/0.435 Test Specimen = 337 APL for anti-Cardiolipin
Reference Values	In a study conducted by , 113 normal donor sera from Northeastern United States were evaluated for Cardiolipin IgA autoantibodies. Of the 113 tested, 1/113 (0.9%) was positive, 1/113 (0.9%) was equivocal, and the remainder 111/113 (98%) were negative for anti-Cardiolipin antibody. In the same study a group of 28 uncharacterized SLE specimens were evaluated for Cardiolipin IgA autoantibodies. Of these 28 specimens, 16/28 (57%) were positive, 3/28 (11%) equivocal, and 9/28 (32%) were negative.
General Description	Autoantibodies directed against phospholipids, and anti-cardiolipin in particular, have been associated with recurrent thrombosis, thrombocytopenia, and spontaneous abortions. aCL is observed in patients with systemic lupus erythematosus, in patients with other connective tissue diseases, in individuals undergoing chlorpromazine treatment, as well as in persons who do not have chronic illness.
Interpretation of Results	Patient samples may be graded as normal, low positive, moderate, or high positive according to the following recommendations: GPL Normal **<>** Low Positive 20 to **<>** Moderate 30 to **<>** High Positive ≥ 80
Reproducibility	To evaluate both intra-assay and inter-assay reproducibility, six specimens were tested; eight replicates each, on each of three days. These results were then used to calculate mean unit values, standard deviations, and percent CV. Two of the specimens were strong positives, two were clearly negative, and two were near the assay cut off.
Limitations	1. A diagnosis should not be made on the basis of anti-Cardiolipin ELISA results alone. Test results for anti-Cardiolipin should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures. 2. The performance characteristics of this device have not been established for lipemic, hemolyzed and icteric specimens; therefore, these specimens should not be tested with this assay. 3. Although aCL has been associated with certain SLE subsets, the clinical significance of aCL in SLE and other diseases remains under investigation. 4. The range of "normal" aCL values may vary from population to population. The normal ranges shown above are those recommended by the manufacturer and are supported by studies of random blood donors from three geographic areas in the United States. Testing laboratories, however, are encouraged to establish normal ranges for their regions. 5. The clinical significance of any test result depends upon its relationship to other medical patient data. Disease diagnosis and management should be based on an evaluation of all relevant patient information.

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