Version 19-04/24

User's Manual

Human Cardiolipin IgM ELISA Kit

REF **DEIA1709**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Cardiolipin Antibody Test System is an enzyme-linked immunosorbent assay (ELISA) designed for the semi-quantitative measurement of circulating IgM autoantibodies to cardiolipin. For research use only.

General Description

Autoantibodies directed against phopholipids, and anti-cardiolipin (aCL) in particular, have been associated with recurrent thrombosis, thrombocytopenia, and spontaneous abortions (1, 2, 3). aCL is observed in patients with systemic lupus erythematosus, in patients with other connective tissue disease (4), in individuals undergoing chlorpromazine treatment (5), as well as in persons who do not have chronic illness.

Principles of Testing

The Cardiolipin IgM ELISA test system is designed to detect IgM 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 antibodies 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 IgM (μ-chain specific) 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 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 original test sample.

Reagents And Materials Provided

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label.

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 IgM (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 phosphate-buffered-saline, (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 ≤15% (w).
8. **Stop Solution:** One 15 mL bottle (red cap) containing 1M H_2SO_4, 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.

The following components are not kit lot number dependent and may be used interchangeably with this ELISA assay: TMB, Stop Solution.

Note: Kit also contains:

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

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 200 µl.
3. Multichannel pipette capable of accurately delivering (50-200 µl)
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.)

Storage

1. Store the unopened kit at 2°C and 8°C.
2. **Coated microwell strips:** Store between 2°C 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°C and 8°C. DO NOT FREEZE.
4. **Calibrator, Positive Control and Negative Control:** Store between 2°C and 8°C.
5. **TMB:** Store between 2°C and 8°C.
6. **Wash Buffer concentrate (10X):** Store between 2°C and 25°C. Diluted wash buffer (1X) is stable at room temperature (20°C - 25°C) for up to 7 days or for 30 days between 2°C and 8°C.
7. **Sample Diluent:** Store between 2°C and 8°C.

8. **Stop Solution:** Store at 2°C and 25°C.

Specimen Collection And Preparation

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. 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.
3. 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.
4. 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°C 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.

Assay Procedure

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°C and 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Controls	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL 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 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 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 300-350 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.

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 50µL 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 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.

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	≤ 0.25
Positive Calibrator	≥ 0.30
Positive Control	≥ 0.50

a. The OD of the negative control divided by the mean OD of the positive calibrator should be ≤ 0.9.

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 < 20 MPL.
   b. The positive control must be> 20 MPL.
6. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Calculation

1. Positive Calibrator

Based upon testing of normal and disease-state specimens, a maximum normal unit 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 MPL

The conversion of OD to unit value (MPL) can be represented by the following equation:

Test Specimen MPL = (A x B)/C

Where: MPL = Unknown unit value to be determined.

A = OD of test specimen in question

B = Unit value of calibrator (MPL).

C = The mean OD of calibrator.

Example: Test specimen OD for Cardiolipin = 0.946

Calibrator OD for Cardiolipin = 0.435

Calibrator unit value for Cardiolipin= 155 MPL

Test Specimen MPL = (0.946 x 155) / 0.435

Test Specimen = 337 MPL for anti-Cardiolipin

Interpretation Of Results

Patient samples may be graded as normal, low positive, moderate, or high positive according to the following recommendations:

	MPL
Normal	< 20
Low Positive	20 to < 30
Moderate	30 to < 80
High Positive	≥ 80

Reference Values

In a study 113 normal donor sera from Northeastern United States were evaluated for Cardiolipin IgM

autoantibodies. Of the 113 tested, one (0.9%) had results of 11 MPL or greater.

In the same study a group of 28 uncharacterized SLE specimens were evaluated for Cardiolipin IgM autoantibodies. Of these 28 specimens, 4 (14.3%) had results of 11 MPL or greater.

Performance Characteristics

Comparative Study An in-house comparative study was performed to demonstrate the equivalence of the Cardiolipin IgM ELISA (DEIA1709) test system to another commercially available Cardiolipin IgM ELISA test system. Performance was evaluated using 260

specimens as described in Table 1 below. The results of the investigation have been summarized in Table 2 below.

Table 1. Summary of Clinical Specimens

No.	Comments
105	Disease state specimens obtained from rheumatology groups from two different university hospitals.
14	Specimens previously tested and found positive for anti-Cardiolipin
28	Uncharacterized SLE patient samples.
113	Normal donor samples collected in Northeastern United States.

Table 2. Calculation of Relative Sensitivity, Specificity, and Agreement

	Commercial Cardiolipin IgM ELISA Result			
		+	-	Totals
DEIA1709 Cardiolipin IgM ELISA Test System	+	44	50	94
	-	3	163	166
	Totals	47	213	260

Relative Sensitivity = 44/47 = 93.6%

95% Confidence Interval* = 86.6 to 100%

Relative Specificity = 163/213 = 76.5%

95% Confidence Interval* = 70.8 to 82.2%

Relative Agreement = 207/ 260 = 79.6%

95% Confidence Interval* = 74.7 to 84.5%

* 95% confidence intervals calculated using the exact method.

Precautions

1. For research use only.
2. 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 protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state and federal laws.
3. The well of the ELISA plate do not contain viable organisms. However, the strips should be considered **potentially biohazardous materials** and handled accordingly.
4. 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 Institutes of Health manual "Biosafety in Microbiology and Biomedical Laboratories", current edition, and OSHA Standard for Bloodborne Pathogens.

5. 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.
6. 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.
7. The human serum Controls, Sample Diluent, Conjugate, and Wash Buffer concentrate contain a preservative (thimerosal, 0.04% (w/v) which may be toxic if ingested.
8. The Stop Solution is **toxic**. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB solution is **harmful**. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an **irritant**. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints which can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. 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.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash solutions should be collected in a disposable basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acidic pH should be neutralized before adding to bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. 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.
26. 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.

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