INSTRUCTIONS FOR USE

EN

Anti-Cardiolipin IgM

REF 2Z51051M
SM2Z51051M

IVD CE Rx Only Σ 96

INTENDED USE

The Anti-Cardiolipin IgM is an ELISA-based test system designed for the semi-quantitative measurement of circulating IgM autoantibodies to Cardiolipin. This test is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Autoantibodies directed against phospholipids, and anti-cardiolipin (aCL) in particular, have been associated with recurrent thrombosis, thrombocytopenia, and spontaneous abortions (1, 2, and 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.

PRINCIPLE OF THE ASSAY

The Anti-Cardiolipin IgM is designed to detect IgM class antibodies to Cardiolipin in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption 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 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.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE:** The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator and Sample Diluent.

Kit Component	Quantity Σ 96	Description
PLATE	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.
CONJ	1	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgM (blue solution). 15mL, white-capped bottle. Ready to use.
CTRL +	1	Positive Control (Human Serum): 0.35mL, red-capped vial. 21X concentrate.
CAL	1	Calibrator (Human Serum): 0.5mL, blue-capped vial. 21X concentrate.
CTRL -	1	Negative Control (Human Serum): 0.35mL, green-capped vial. 21X concentrate.

DIL SPE	1	Sample Diluent: 30mL, green-capped, bottle containing bovine serum albumin and phosphate-buffered-saline. Green solution, ready to use.
SOLN TMB	1	TMB: 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	1	Stop Solution: 15mL, red-capped bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASH 10X	1	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTE: The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB and Stop Solution.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel 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. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e., 10% household bleach - 0.5% sodium hypochlorite).

STORAGE CONDITIONS

2°C – 8°C	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening, strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remain blue.
	Conjugate – DO NOT FREEZE.
	Unopened Kit, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent.
2°C – 25°C	Stop Solution: 2 – 25 °C Wash Buffer (1X) : 20 - 25°C for up to 7 days, 2 - 8°C for 30 days Wash Buffer (10X): 2 - 25°C

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. 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.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The 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, handle these products 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 Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (6).

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 Sample Diluent, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
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. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a 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 that 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 bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (7, 8). Do not use if there are any added anticoagulants or preservatives. 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 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (10).

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 for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank 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 - 8°C.

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

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. 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 Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 washes.
      4. 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 disinfectant at the end of the day's 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 to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
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 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the ssame order as the TMB. 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. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. → *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. → *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. → *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the 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, Positive Control, and Negative Control should fall within the following ranges:

	OD Range
Negative Control	≤0.250
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, but will not ensure precision at the assay Cutoff.
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.
7. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations:**
   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 for the determination of 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. **Interpretations:** Patient samples may be graded as normal, low positive, moderate, or high positive according to the following recommendations:

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

2. **Conversion of Optical Density to MPL**: The conversion of OD to unit value (MPL) can be represented by the following equation:

$$\text{Test Specimen MPL} = (A \times B)/C$$

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

LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis based on the Anti-Cardiolipin IgM results alone. Interpret the test results for anti-Cardiolipin in conjunction with clinical evaluation and results of other diagnostic procedures.
2. The performance characteristics of this device have not been established for lipemic, hemolyzed and icteric specimens; therefore, do not use these type specimens with this assay.

3. Although aCL has been associated with certain SLE subsets (1 - 3), 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. Base disease diagnosis and management on an evaluation of all relevant patient information.
6. False positive anti-Cardiolipin IgM results have been reported due to the presence of rheumatoid factor IgM and anti-Cardiolipin IgG (9).

EXPECTED RESULTS

A study was conducted evaluating 113 normal donor sera, from the Northeastern United States, 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 for Cardiolipin IgM autoantibodies was also evaluated. Of these 28 specimens, four (14.3%) had results of 11 MPL or greater.

PERFORMANCE CHARACTERISTICS

1. Comparative Study

A comparative study was conducted to demonstrate the equivalence of the Anti-Cardiolipin IgM 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

Quantity (n)	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: Relative Sensitivity, Specificity, and Agreement

		Commercial Cardiolipin IgM ELISA Result		
		Positive	Negative	Total
Anti-Cardiolipin IgM	Positive	44	50	94
	Negative	3	163	166
	Total	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.

2. 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 cutoff. The results of the study have been summarized below.

Table 3: Anti-Cardiolipin IgM; Results of Precision Testing

	Intra-Assay									Inter- Assay		
	Day One			Day Two			Day Three					
Specimen	Mean Result (MPL)	Standard Deviation	% CV	Mean Result (MPL)	Standard Deviation	% CV	Mean Result (MPL)	Standard Deviation	% CV	Mean Result (MPL)	Standard Deviation	% CV
1	248	6.30	2.5	229	13.48	5.9	220	13.2	6.0	232	16.1	7.0
2	157	5.62	3.6	139	3.43	2.5	146	6.72	4.6	147	9.1	6.2
3	58	0.84	1.4	51	2.6	5.1	32	3.46	10.8	47	11.4	24.3

4	42	2.14	5.1	39	1.87	4.8	43	1.80	4.2	41	2.5	6.1
5	5	0.36	7.6	5	1.31	28.4	4	3.28	75.3	5	2.0	43.0
6	0	0.67	-52.1	0	0.61	580.6	0	0.29	82.8	0	0.9	-324.2

3. **Cross Reactivity:**
To investigate the potential for positive reactions due to cross reactive antibodies, 14 specimens which were reactive for various autoantibodies were tested on the Anti-Cardiolipin IgM. None of the 14 were positive for anti-Cardiolipin IgM antibody. The results of this study indicate that the potential for a high degree of cross reactivity with such autoantibodies is not likely.

REFERENCES

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GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	PLATE	Plate
REF	Catalogue number	CONJ	Conjugate
Σ n	Sufficient for *n* tests	CTRL +	Positive Control
LOT	Batch code	CTRL -	Negative Control
	Use by	CAL	Calibrator
	Temperature limitation	DIL SPE	Sample Diluent
CONT	Contents	SOLN TMB	TMB
UDI	Unique Device Identifier	SOLN STOP	Stop Solution
	Consult the warnings and precautions	WASH 10X	Wash Buffer Concentrate (10X)
	Consult electronic instructions for use	EN	English
	Store in the upright position	Made in the USA	Made in the USA
RX Only	Applicable for U.S.A: Prescription *in vitro* diagnostic product		Corrosive
	Hazardous Communication	EC REP	European Commission Authorized Representative
	Conformity with Directive 98/79		

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