

IVD



See external label

2°C-8°C



Σ=96 tests

REF

cat. #1490-1

CARDIOLIPIN IgG,A,M ELISA

Catalog No. 1490-1

For *in vitro* diagnostic use. High Complexity test.

INTENDED USE

The Diagnostic Automation. Cardiolipin Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection and qualitative determination of IgG, IgA, and IgM antibodies to cardiolipin in human sera. The assay is to be used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid in the diagnosis of the anti-phospholipid syndrome in patients with autoimmune disease. For *in vitro* diagnostic use.

INTRODUCTION

In patients with Systemic Lupus Erythematosus (SLE), antibodies to cardiolipin (a negatively charged phospholipid) have been associated with both arterial and venous thrombosis, thrombocytopenia, and recurrent fetal loss (1,2,3). Patients with the anti-cardiolipin syndrome have one of the above clinical features and have antibodies to cardiolipin and/or a positive lupus anticoagulant test (4). The antibodies present to cardiolipin may be of the IgG, IgA, IgM isotypes (5,6,7). Testing for the various antibody isotypes to cardiolipin aid in diagnosis of the anti-phospholipid syndrome in patients with SLE or lupus-like disorders. Several Enzyme-Linked Immunosorbent Assays have been developed and validated for detecting antibodies to cardiolipin (8,9).

PRINCIPLE OF THE TEST

The DAI Cardiolipin IgG, IgA, IgM test is an Enzyme-Linked Immunosorbent Assay for the detection and qualitative determination of IgG, IgA, and IgM antibodies to cardiolipin antigens. Purified cardiolipin antigen is attached to a solid phase microassay well. Diluted test sera are added to each well. If the antibodies are present that recognize the antigen, antigen-antibody complexes are formed. After incubation, the wells are washed to remove unbound antibody. An enzyme labeled anti-human IgG,A,M is added to each well. If antibody is present, the conjugate will bind to the antigen-antibody complexes. After incubation, the wells are washed to remove unbound conjugate. A substrate solution is added to each well. If enzyme is present, the substrate will undergo a color change. After an incubation period, the reaction is stopped and the color intensity is measured photometrically, producing an indirect measurement of specific antibody in the patient specimen (15, 16, 17, 18).

KIT COMPONENTS

1. **Cardiolipin antigen coated microassay plate:** 96 wells, provided with a strip holder and stored in a foil pouch with desiccant.
2. **Wash Buffer (20X concentrate):** One bottle, 50 mL. Contains buffer and Tween 80, and 0.1% Proclin as a preservative. Dilute 1 part concentrate + 19 parts deionized or distilled water.
3. **Serum Diluent:** One bottle, 30 mL. Contains buffer, BSA and 0.1% Proclin as a preservative.
4. **Horse Radish Peroxidase (HRP) Conjugate:** One bottle, 15 mL. Goat anti-human IgG, IgA, and IgM containing proclin (0.1%) and gentamicin as preservatives). Ready to use.
5. **Chromogen/Substrate Solution:** One bottle, 15 mL. Contains 3,3',5,5' - tetramethylbenzidine (TMB). Should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
6. **Stop Solution:** One bottle, 15 mL. Contains 1N H₂SO₄ solution.
7. **High Positive Control:** One vial, 0.4 mL, human serum, containing <0.1% sodium azide and <0.01% pen/strep as preservatives. Established range printed on vial label. High Positive Control used to control the upper dynamic range of the assay.
8. **Negative Control:** One vial, 0.4 mL, human sera, containing <0.1% sodium azide and <0.01% pen/strep as preservatives, established range printed on vial label. Negative control used to control the negative range of the assay.
9. **Low Positive Control:** One vial 0.4 mL, human serum, containing <0.1% sodium azide and <0.01% pen/strep as preservatives, established range printed on vial label. Low Positive Control used to control the range near the cutoff of the assay.
10. **Calibrator:** One vial, 0.4 mL, human serum, containing <0.1% sodium azide and <0.01% pen/strep as preservatives, with Kit specific Correction Factor printed on vial label. Calibrator used to calibrate assay to account for day-to-day fluctuations in temperature and other testing conditions.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 μ L volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tubes for serum dilutions.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water, CAP Type 1 or equivalent.
9. Timer capable of measuring to an accuracy of +/- 1 second.
10. Disposal basin and 0.5% sodium hypochlorite (50 mL bleach in 950 mL H₂O).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specification of the reader.

STORAGE AND SHELF LIFE OF REAGENTS

1. All kit components that are stored at their recommended storage conditions are stable until the expiration date on their label. Do not use past their expiration date.
2. Antigen coated wells. Unused strips should be immediately resealed in the foil bags with desiccant/humidity indicator and returned to storage at 2°- 8° C. If the bag is resealed with tape the wells are stable for 30 days. If the bag is resealed with a heat sealer the wells are stable until their expiration.
3. All other reagents are stored at 2°- 8° C in their original containers.
4. Store 1X (diluted) Wash Buffer at room temperature (21° to 25° C) for up to 5 days, or 1 week between 2°- 8° C.

PRECAUTIONS

1. Each donor unit in the preparation of the Calibrator and Controls was tested by an FDA approved method for the presence of the antibody to HIV-1 as well as for hepatitis B surface antigen and found to be negative. Because no test method can offer complete assurance that human immunodeficiency virus (HIV-1), hepatitis B virus, or other infectious agents are absent, these specimen/reagents as well as patient samples, 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 Microbiology and Biomedical Laboratories," 1993 (12).
2. Certain reagents in this kit contain sodium azide for use as a preservative. Azides may react with lead and copper plumbing to form explosive azide compounds. When disposing of reagents, flush with copious quantities of water to minimize azide build up.
3. This product is for **IN VITRO DIAGNOSTIC USE** only.
4. Reagents contain preservatives which may be toxic if ingested.
5. Do not pipette by mouth. Avoid contact of reagents and patient specimens with skin or mucous membranes.
6. Do not allow the stop solution to contact skin or eyes. If contact occurs, immediately flush with copious quantities of water.
7. Avoid splashing or generation of aerosols.
8. Do not use heat inactivated sera.
9. Do not mix or interchange reagents between lots of kits or from other manufacturers.
10. Do not dilute or adulterate kit reagents.
11. Do not cross contaminate reagents or specimens.
12. Do not use TMB Substrate solution if it has begun to turn blue.
13. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
14. Do not vary reagents and incubation temperatures above or below room temperature (21°- 25° C).
15. Washing is important. Improperly washed wells will give erroneous results. Do not allow the wells to dry out between incubations.

SPECIMEN COLLECTION

1. Aseptically collect blood samples by venipuncture and prepare serum using accepted technique (13).
2. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
3. Sera may be stored up to five days at 2°- 8° C. If a further delay in testing is needed, store frozen at -20° to -70°C in a non-defrosting freezer. Avoid multiple freeze/thaw of patient samples.
4. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
5. Do not heat inactivate sera.

PREPARATION OF REAGENTS

1. All reagents must be removed from refrigeration and allowed to come to room temperature (21°- 25° C) before use. Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer to 1L with distilled and/or deionized H₂O. Mix well.

Note: Wash Buffer may form crystals at 4° C. If crystals are present, the Wash Buffer must be warmed to 37° C in a waterbath or incubator before use.

GENERAL PROCEDURE

1. Remove the number of wells needed from the plate pouch and arrange in a strip holder. The remaining strips should be resealed in the plate pouch with desiccant/humidity indicator. The pouch should be reheat sealed or rolled over and the end taped. If the color of the desiccant/humidity indicator changes from blue to pink, the strips should not be used. Determine the number of patients to be assayed. For each assay, the Calibrator should be run in triplicate. Also the High Positive Control, Low Positive Control, Negative Control, and a reagent blank should be run on each assay. Check software and reader requirements for the correct Calibrator/Control configurations.
Example Configuration:

1A	RB	2A	Patient #2
1B	NC	2B	Patient #3
1C	Cal	2C	Patient #4
1D	Cal	2D	Patient #5
1E	Cal	2E	Patient #6
1F	HPC	2F	Patient #7
1G	LPC	2G	Patient #8
1H	Patient #1	2H	Patient #9

RB = Reagent Blank – Well without serum addition run with all reagents. Utilized to blank reader.

NC = Negative Control

Cal = Calibrator

HPC = High Positive Control

LPC = Low Positive Control

2. Dilute test sera, Calibrator and Control sera, prepare a 1:21 i.e., 10 μ L of each serum sample to 200 μ L of Serum Diluent. Mix well.(For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patients serum.
3. Transfer 100 μ L of the prediluted samples to the reaction wells, using a multichannel pipette. Withdraw and expel each sample at least three times to ensure proper mixing of the sample before transferring to the reaction plate. Use new fresh pipette tips for each sample. Add 100 μ L of Serum Diluent to the reagent blank.
4. Incubate each well of the reaction plate at room temperature (21°- 25° C) for 30 minutes \pm 1 minute.
5. Wash the reaction plate three times with 1X Wash Buffer. Shake all of the liquid out of the wells. With a wash bottle, automated or semi-automated wash system fill each well with 250-300 μ L Wash Buffer making sure no air bubbles are trapped in the wells. Shake all of the Wash Buffer out of the wells. Repeat the wash two more times. A total of up to 5 washes may be necessary with automated equipment. After the last wash, shake out the Wash Buffer and remove residual Wash Buffer by tapping the plate firmly on a paper towel. The Wash Buffer can be collected in a basin and treated with 0.5% sodium hypochlorite (bleach) at the end of the days run.
6. Add 100 μ L of the Conjugate to each well of the reaction plate, including reagent blank.
7. Incubate each well of the reaction plate at room temperature (21°- 25° C) for 30 minutes \pm 1 minute.
8. Repeat wash as described in Step 5.
9. Add 100 μ L of the Chromogen/Substrate Solution to each well of the reaction plate, including reagent blank.
10. Incubate each well of the reaction plate at room temperature (21°- 25°C) for 15 minutes \pm 1 minute.
11. Add 100 μ L of the Stop Solution to each well, including reagent blank, at the same rate as the TMB Substrate was added. Positive samples will turn from blue to yellow. Tap plate to ensure mixing. Wait a minimum of 5 minutes and read.
12. Read the plate using a spectrophotometer at a wavelength of 450 nm. If dual wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450nm. If the reagent blank is \geq 0.150 the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Measure each optical density (OD) against the reagent blank. The plate should be read within 30 minutes of assay completion. Dispose of the plates after readings have been obtained.

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:

1. Calibrator and Controls must be run with each test run.
2. Reagent Blank must be $<$ 0.15 O.D. (Optical Density) at 450 nm when read against air blank.
3. The mean O.D. Value for the Calibrator should be \geq 0.300 at 450 nm when read against the reagent blank.
4. The Index Values for the High, Low, and Negative Control should be in their respective ranges printed on the vials. If the control values are not within their respective ranges, the test should be considered invalid and should be repeated.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Refer to NCCLS C24A for guidance on appropriate Quality Control practices (19).
6. If above criteria are not met on repeat, contact DAI Technical Service.

CALCULATION OF RESULTS

1. Calibrator Value - Calculate the mean value for the Calibrator from the three Calibrator determinations. If any of the three Calibrator Values differ by more than 15% from the mean, discard that value and calculate using the mean of the two remaining values.
2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by DAI for each lot of kits. The Correction Factor is printed on the Calibrator vial.
3. Cutoff O.D. Value - The Cutoff O.D. value for each assay is determined by multiplying the Correction Factor by the mean Calibrator Value determined in step 1.
4. Index Value - Calculate an Index Value for each specimen by dividing the specimen O.D. Value by the Cutoff O.D. determined in step 3.

Example : O.D.s obtained for Calibrator = 0.38, 0.40, 0.42

Mean O.D. for Calibrator	= 0.40
O.D. obtained for patient sera	= 0.60
Correction Factor	= 0.50
Cutoff Value	= 0.50 x 0.40 = 0.20
Index Value	= 0.60/0.20 = 3.00

Analysis

1. The patients' index Values are interpreted as follows:

<u>Index Value</u>	<u>Interpretation</u>
≤ 0.90	Negative
0.91 - 1.09	Equivocal
≥ 1.10	Positive

2. Specimens with Index Values in the equivocal range should be retested. If still equivocal, retest by an alternate method or test a new sample.

EXPECTED VALUES

1. From 2-10% of apparently normal individuals may contain antibodies to cardiolipin (2).
2. Approximately 44% of patients with SLE have antibodies to cardiolipin (2).
3. From 50-75% of SLE patients with cardiolipin antibodies have one or more episodes of thrombosis, thrombocytopenia or fetal loss (2).

LIMITATIONS

1. The result of the assay should not be interpreted as being diagnostic. The results should only be used as an aid to diagnosis. The results should be interpreted in conjunction with the clinical evaluation of the patient.
2. Sera from patients with other autoimmune diseases (12%) and from normal individuals (2%) may contain antibodies to cardiolipin (10).
3. Many syphilis patients will have antibodies to cardiolipin (2).
4. Antibodies to cardiolipin can be present during many (32%) acute bacterial infections (11).
5. The assay should be used only with serum. Icteric, lipemic, hemolyzed and heat inactivated serum should be avoided.
6. The assay has been validated (see Table 3) to be linear with dilution up to an Index Value of 4.99. Sera with values greater than 4.99 should be reported as > 4.99.
7. Specimens with Index Values in the equivocal range should be retested. If still equivocal, retest by an alternate ELISA method or test a new sample.

PERFORMANCE CHARACTERISTICS **SENSITIVITY AND SPECIFICITY**

The DAI Cardiolipin IgG,A,M ELISA kit was evaluated relative to a commercially available ELISA test kit. Table 1 summarizes the data.

Table 1
Sensitivity and Specificity of the DAI Cardiolipin ELISA Kit

		DAI CARDIOLIPIN ELISA KIT			Total
		Positive ≥ 1.10	Equivocal 0.91-1.09	Negative ≤ 0.90	
Alternate ELISA KIT	Positive	35	0	0	35
	Negative	2	1	128	131
	Total	37	1	128	166

Sera falling in the equivocal range were excluded from the following calculations.

Relative Sensitivity = 35/35	= 100%
Relative Specificity = 128/130	= 98.5 %
Relative Accuracy = 163/165	= 98.8%

PRECISION

The precision of the DAI Cardiolipin IgG,A,M ELISA kit was determined by testing seven different sera eight times each on three different assays. The data are summarized in Table 2. With proper technique, the user should obtain C.V.'s of less than 20%.

Table 2
CARDIOLIPIN Precision Data

	Assay 1 (n=8)			Assay 2 (n=8)			Assay 3 (n=8)			Inter Assay (n=24)		
	X	S.D.	C.V.	X	S.D.	C.V.	X	S.D.	C.V.	X	S.D.	C.V.
1	2.73	0.335	12.27%	2.55	0.341	13.37%	3.17	0.418	13.19%	2.81	0.441	15.69%
2	1.99	0.238	11.96%	1.89	0.161	8.52%	2.25	0.423	18.80%	2.04	0.322	15.78%
3	1.87	0.157	8.40%	1.84	0.153	8.32%	1.94	0.216	11.13%	1.88	0.175	9.31%
4	2.38	0.272	11.43%	2.27	0.318	14.01%	2.77	0.397	14.33%	2.47	0.386	15.63%
5	1.16	0.110	9.48%	1.18	0.081	6.86%	1.29	0.117	9.07%	1.21	0.116	9.59%
6	0.09	0.024	26.67%	0.14	0.025	17.86%	0.17	0.044	25.88%	0.15	0.034	22.67%
7	0.31	0.051	16.45%	0.34	0.051	15.00%	0.39	0.070	17.95%	0.35	0.065	18.57%

X = Mean Cardiolipin Value
S.D. = Standard Deviation
C.V. = Coefficient of Variation

LINEARITY

The DAI Cardiolipin IgG,A,M Index Values were determined for serial twofold dilutions of five positive sera. The Index Values were compared to log₂ of dilution by standard linear regression.

Table 3
Linearity

Serum #	Neat	1:2	1:4	1:8	1:16	r
1	2.45	1.55	0.71			0.999
2	3.10	1.66	0.92			0.983
3	4.37	2.68	1.71	0.89		0.985
4	2.37	1.15	0.54			0.982
5	4.99	2.87	1.80	1.00	0.51	0.962

r = correlation coefficient.

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