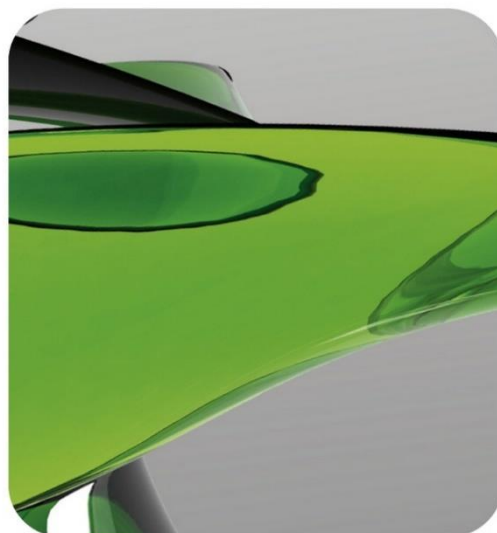




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INSTRUCTION MANUAL

AESKULISA® Cardiolipin-GM

REF 7204US





Product Ref.	7204US
Product Desc.	Cardiolipin-GM
Manual Rev. No.	008: 2026-01-14

Instruction manual

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1 Intended Use

AESKULISA® Cardiolipin-GM is a solid phase enzyme immunoassay employing highly purified cardiolipin plus native **human** β 2-glycoprotein I for the semi-quantitative and qualitative detection of IgG and/or IgM antibodies against cardiolipin in human serum. Anti-cardiolipin antibodies mainly recognize specific epitopes on a complex composed out of cardiolipin and β 2-glycoprotein I which are expressed only when β 2-glycoprotein I interacts with cardiolipin.

The assay is an aid in the diagnosis of systemic lupus erythematosus (SLE), primary and secondary anti-phospholipid syndrome (APS) and should be used in conjunction with other serological tests and clinical findings.

2 Clinical Application and Principle of the Assay

Antibodies against Cardiolipin belong to the group of anti-phospholipid antibodies specific for negatively charged phospholipids, components of biological membranes. Cardiolipin is an acidic phospholipid derived from glycerol and was named because of its isolation from bovine heart in 1941. Anti-phospholipid antibodies are frequently found in sera of patients with systemic lupus erythematosus (SLE) and related diseases. The prevalence of anti-cardiolipin antibodies in SLE is 24-50 %.

The occurrence of anti-cardiolipin antibodies in patients with SLE and related diseases is typical for a secondary anti-phospholipid syndrome (APS). In contrast, anti-cardiolipin antibodies in patients with no other autoimmune diseases characterize the primary anti-phospholipid syndrome (APS). Many studies have shown a correlation between these autoantibodies and an enhanced incidence of thrombosis, thrombocytopenia and habitual abortions (as a consequence of a placental infarct). The exact mechanism by which pathogenic anti-phospholipid antibodies induce thrombosis is not yet revealed fully.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.



3 Kit Contents

To be reconstituted:

- 5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
Containing: Tris, NaCl, BSA, sodium azide < 0.1 % and thimerosal 0.01% (preservative)
- 50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide < 0.1 % and thimerosal 0.01% (preservative)

Ready to use:

- Negative Control 1 vial, 1.5 ml (capped green: yellow solution)
Containing: Human serum (diluted), sodium azide < 0.1 % (preservative)
- Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
Containing: Human serum (diluted), sodium azide < 0.1 % (preservative)
- Cut-off control 1 vial, 1.5 ml (capped blue: yellow solution)
Containing: Human serum (diluted), sodium Azide < 0.1 % (preservative)
- Calibrators 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml
(color increasing with concentration: yellow solutions)
Containing: Human serum (diluted), sodium azide < 0.1 % (preservative)
- Conjugates 1 vial, 15 ml IgG (capped blue: blue solution)
1 vial, 15 ml IgM (capped green: green solution)
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase and thimerosal < 0.01 % (preservative)
- TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H₂O₂
- Stop Solution 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid
- Microtiterplate 12x8 well strips with breakaway microwells
Coating see paragraph 1

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware, test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette. Microplate washing device (multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia and the European Pharmacopeia.

4 Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35.6-46.4°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 2-8°C/35.6-46.4°F, at least.

Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.



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5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though the kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

Do not smoke, eat or drink when manipulating the kit.

Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus, handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-26°C/68-78.8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Never expose components to higher temperature than 37°C/ 98.6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic and medicinal methods if the patient has got infectious diseases accompanied by medication.

6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8°C/35.6-46.4°F up to three days, or frozen at -20°C/-4°F for longer periods.

7 Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples

Dilute serum samples 1:101 with sample buffer (1x).

e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well!

Washing

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35.6-46.4°F).

7.2 Work flow

For pipetting scheme see Annex A, for the test procedure see Annex B

NOTE: If IgG and IgM are determined in parallel, calibrators, controls and samples have to be done twice, for each subclass separately.

- Pipette 100 µl of each patient's diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off control and negative and positive controls into the designated wells.
- Incubate for 30 minutes at room temperature (20-26°C/68-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/68-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/68-78.8°F), in the dark.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8 Quantitative and Qualitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in **U/ml (x-axis)**. For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in **U/ml**.

Normal Range	Positive Results
≤ 15 U/ml	>15 U/ml

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators IgG	OD 450/620 nm	CV % (Variation)
0 U/ml	0.066	3.2
3 U/ml	0.162	0.4
10 U/ml	0.291	1.7
30 U/ml	0.597	1.3
100 U/ml	1.101	2.9
300 U/ml	2.039	0.4

Example of a calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.772/0.752	0.762	48.8
P 02	1.058/1.038	1.048	82.9

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations. **Do not use this example for interpreting patients results!**

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

For **qualitative interpretation** read the optical density of the cut-off control and the patient samples. Compare patient's OD with the OD of the cut-off control. All samples which are higher than cut-off are considered positive.

Negative: OD patient < OD cut-off Positive: OD patient > OD cut-off
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9 Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	60 minutes at room temperature (20-26°C/68-78.8°F)
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C/35.6-46.4°F use original vials, only
Number of determinations:	96 tests

10 Performance Data

10.1 Analytical Sensitivity

The analytical sensitivity of this kit has been found at 1.0 U/ml.

10.2 Specificity and sensitivity

The microplates is coated with **highly purified cardiolipin and native human β2-glycoprotein I**. No cross reactivities to other autoantigens have been found.

Cardiolipin antibodies are detected in up to 50% of SLE and 80-90% of APS patients (2).

A study with 111 sera (28 SLE, 19 primary APS, 15 secondary APS and various other autoimmune diseases) on the **AESKULISA®** Cardiolipin-GM and a predicate device is shown in the table below:

predicate device	AESKULISA Cardiolipin G (M)		
		positive	negative
	positive	42 (18)	4 (22)
	negative	1 (1)	64 (70)

95.5 % (79.3 %) agreement

Please be advised that agreement refers to comparison of the assay's results to that of a similar assay. There was no attempt to correlate the assay's results with that of the disease presence or absence. No judgement can be made on the comparisons accuracy to predict disease.

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	Measured concentration (U/ml)	Expected concentration (U/ml)	Recovery (%)
1	1 / 100	63.1	68.0	93.0
	1 / 200	33.7	34.0	99.1
	1 / 400	15.9	17.0	93.5
	1 / 800	9.0	8.5	105.9
2	1 / 100	138.6	140.8	97.7
	1 / 200	70.1	70.9	98.9
	1 / 400	33.2	35.5	93.5
	1 / 800	17.9	17.7	101.1

10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Sample ID	Intra-Assay Precision		Inter-Assay Precision	
	Mean (U/ml)	CV (%)	Mean (U/ml)	CV (%)
1	586.2	1.5	499.8	0.9
2	67.4	3.4	68.9	1.7
3	34.5	7.6	40.7	4.6

10.5 Calibration

In the absence of an international reference standard, the quantitative measurement system is calibrated in arbitrary units. The results are expressed in U/ml.

11 Literature

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12 Regulatory Symbols

IVD	For in vitro diagnostic use
REF	Catalog number
LOT	Lot
UDI	Unique Device Identifier
	96 tests
	See instructions for use
	Use by
	Store at 2-8°C (35.6-46.4°F)
	Manufactured by
CON +	Positive Control
CON -	Negative Control
CAL	Calibrator
CO-CAL	Cut off Calibrator
CONJ	Conjugate
MP	Coated microtiter plate
WASHB 50x	Wash buffer
SUB	Substrate buffer
STOP	Stop solution
SB 5x	Sample buffer
Rx only	For Prescription Use only



Annex

A: Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For **semi-quantitative interpretation** use calibrators to establish a standard curve

For **qualitative interpretation** use cut-off control

	for semiquantitative interpretation use calibrators to establish a standard curve						for qualitative interpretation use cut-off calibrator					
	1	2	3	4	5	6	7	8	9	10	11	12
A	CalA	CalE	P1				NC	P3				
B	CalA	CalE	P2				NC	P4				
C	CalB	CalF	P3				CC	P5				
D	CalB	CalF	P4				CC	P6				
E	CalC	PC	P5				PC	...				
F	CalC	PC	P6				PC	...				
G	CalD	NC	...				P1	...				
H	CalD	NC	...				P2	...				

CalA: calibrator A, CalB: calibrator B, CalC: calibrator C, CalD: calibrator D, CalE: calibrator E, CalF: calibrator F

PC: positive control

NC: negative control

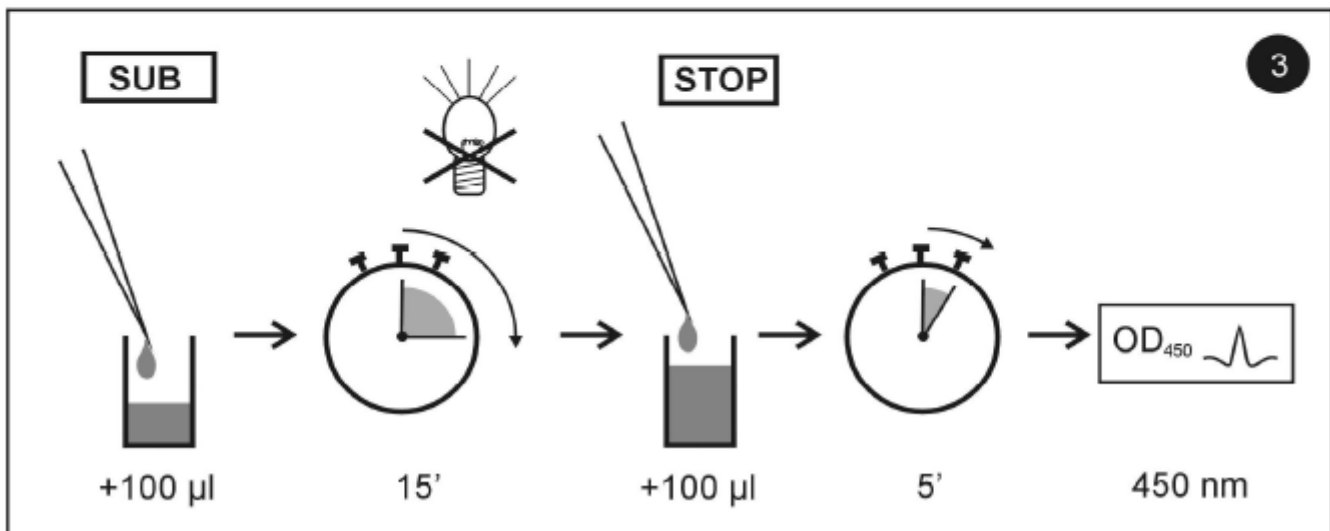
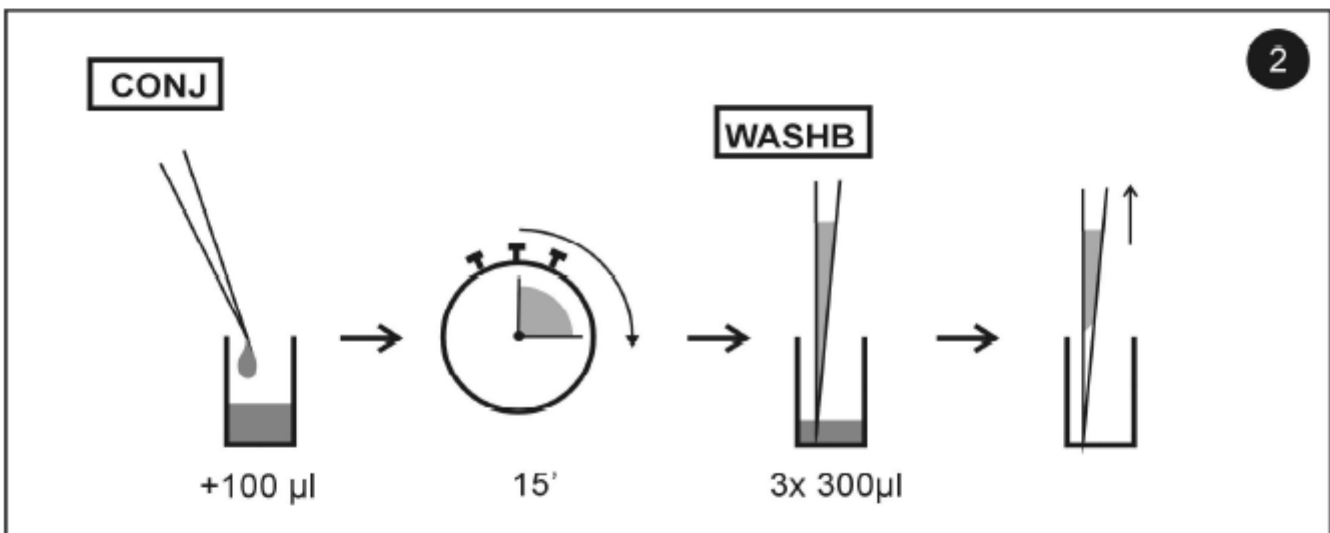
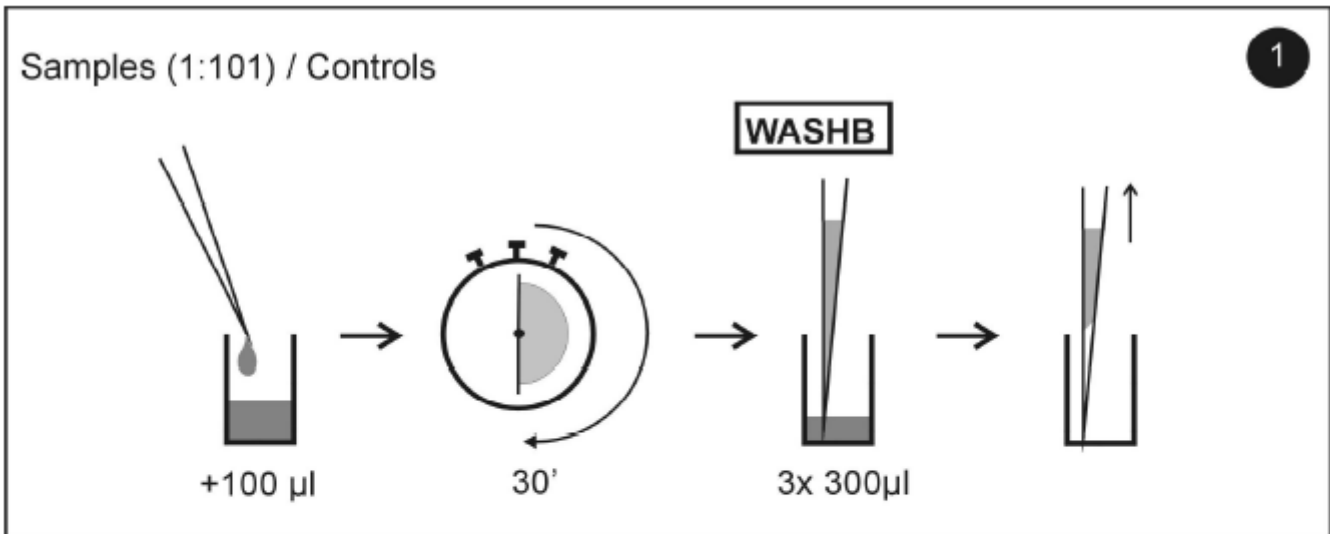
CC: Cut-off calibrator

P1: patient 1

P2: patient 2

P3: patient 3

B: Test Procedure



C: Test Protocol

Assay/Test: _____

Incubation/ Inkub. : 1. _____ min

Date/Datum: _____

Temperature/Temperatur: _____ °F _____ °C

Signature/Unterschrift: _____

Name: _____

2. _____ min
 3. _____ min

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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