



EIASON[®] Anti Cardiolipin IgG/IgM



Enzyme immunoassay for the quantitative determination of IgG and IgM class autoantibodies against cardiolipin in human serum or plasma

Kit instruction

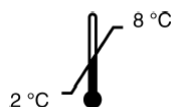
For in-vitro use only

Product of







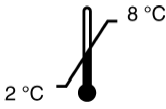











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REF E07-106-96



Used IFU-Symbols

Symbol	English	Symbol	English
	In vitro diagnostic device		Sample Buffer
	Order number		Conjugate IgG Conjugate IgM
	Product of		Wash Buffer
	Storage		Batch code
	European Conformity		Substrate
	Expiry date		Stop Solution
	Calibrators		Positive Control
	microplate		Negative Control

Intended Use

For in-vitro use only.

The EIASON® Anti Cardiolipin IgG/IgM kit is an enzymeimmunoassay intended for the quantitative determination of IgG and IgM class autoantibodies against cardiolipin in human serum or plasma. Results are to be used as an aid in the determination of an increased risk of thrombosis in patients with Systemic Lupus Erythematosus (SLE) or lupus-like disorders. Indication for determination of anti-Cardiolipin antibodies: SLE, Thrombosis, Thrombocytopenia, Cerebral Ischemia, Chorea, Epilepsy, Recurrent Abortion, Intrauterine Death.

Assay principle

Highly purified cardiolipin is bound to microwells saturated with β 2-glycoprotein I. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG and IgM immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG resp. IgM antibodies present in the original sample.

Warnings and Precautions

The EIASON[®] Anti Cardiolipin IgG/IgM kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided.

CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Shelf Life and Storage of Reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.

Storage and preparation of serum samples

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below -20°C. Repeated freezing and thawing or increases in storage temperature must be avoided. Incorrect storage of serum samples can lead to loss of autoantibody activity.

Do not use lipaemic or grossly haemolysed serum samples.

When required, thaw test sera at room temperature and mix gently to ensure homogeneity.

Materials provided

Allow all reagents 1-9 to reach room temperature before use.

1. **MPL** Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified bovine cardiolipin and saturated with β 2-Glycoprotein I. Ready to use. Before opening the packet of strip wells, allow it to stand at room temperature (20-25°C) for at least 30 minutes. After opening, keep any unused wells in the original foil packet (reseal with adhesive tape) and in the self-seal plastic bag with the desiccant provided. Store at 2-8°C and use within 3 months. However, we recommend that strip wells are used on the same day the foil packet is opened. A frame for holding the wells during assays is also provided.
2. **CAL A - F** Combined Calibrators with IgG and IgM class Anti-Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, $\text{NaN}_3 < 0.1\%$ (w/w)) containing: IgG (0; 7.5; 15; 30; 60 and 120 U/ml) and IgM (0; 0.5; 10; 20; 40 and 80 U/ml), exact concentrations see label of each vial, ready to use, 1.5 ml each.
3. **PC** **NC** Positive and Negative Control in a serum/buffer matrix (PBS, BSA, $\text{NaN}_3 < 0.1\%$ (w/w)), exact concentrations see label of each vial, ready to use, 1.5 ml each, 2 vials.
4. **BU** Sample buffer, 1 vial, 20 ml, (TRIS, $\text{NaN}_3 < 0.1\%$ (w/w)), yellow, concentrate 5 x, dilute the vial with distilled water to a final volume of 100 ml prior to use. Stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.
5. **CONJG** Enzyme Conjugate solution (PBS, Proclin 300 $< 0.5\%$ (v/v)), light red, containing polyclonal rabbit anti-human IgG, labelled with horseradish peroxidase, ready to use, 1 vial, 15 ml.
6. **CONJM** Enzyme Conjugate solution (PBS, Proclin 300 $< 0.5\%$ (v/v)), light red, containing polyclonal rabbit anti-human IgM, labelled with horseradish peroxidase, ready to use, 1 vial, 15 ml.
7. **SUB** Peroxidase substrate (tetramethyl benzidine; TMB; 15 ml ready to use).
8. **STOP** Stop solution (contains acid; 15 ml ready to use).
9. **WASH** wash solution (PBS, $\text{NaN}_3 < 0.1\%$ (w/w)), concentrate 50 x. Dilute the vial with distilled water to a final volume of 1000 ml prior to use. Stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

Materials required but not provided in the kit

- Pipettes capable of dispensing 10, 100 and 1000 μ l
- Vortex Mixer
- Distilled water
- ELISA plate reader suitable for 96 well formats and capable of measuring absorbances at 450 and 405 nm

Assay procedure

Calculate the number of individual **MPL** wells needed for the assay. Allow all the reagents supplied including the appropriate number of strips to reach room temperature, fit the number of strip wells required firmly into the frame provided. Kit controls should always be included in each assay run.

1. Dilute all samples 1:100 with **BU** before assay. Therefore combine 10 µl of sample with 990 µl of **BU** in a polystyrene tube. Mix well.
2. Pipette 100 µl of **CAL A - F** **PC** **NC** and diluted test sera into the wells (in duplicate).
3. Incubate for 30 minutes at room temperature (20-25°C).
4. Discard the samples by briskly inverting the frame of strip wells over a suitable receptacle. Wash 3 times with 300 µl **WASH** per well and each time tap the inverted wells gently on a clean dry absorbent surface to remove any droplets of wash buffer.
5. Pipette carefully 100 µl either **CONJG** or **CONJM** into each well.
6. Incubate for 15 minutes at room temperature (20-25°C).
7. Discard the **CONJG** or **CONJM** by briskly inverting the wells over a suitable receptacle, wash 3 times with **WASH** as described under point 4.
8. Pipette carefully 100 µl of **SUB** into each well.
9. Incubate for 15 minutes at room temperature (20-25°C).
10. Stop the substrate reaction by careful addition of 100 µl of **STOP** to each well (this will cause the blue colour to turn yellow) and shake the plate for about 5 seconds on a plate shaker to ensure uniformity of the solution in each well. It is most important to ensure that the substrate incubation time (i.e. time from addition of **SUB** to addition of **STOP**) is the same for each well.
1. Measure the absorbance of each well at 450 nm and 405 nm for overrange filter (reference 620 – 650 nm) within 5 minutes after adding the stop solution.

or fully automated on:

- **IASON® PersonalLab**
- **IASON® Quardette**
- **IASON® Gladiator**

Quality Control

EIASON® Anti Cardiolipin IgG/IgM test is only valid if the optical density at 450 nm for Negative Control **NC** and Positive Control **PC** as well as for the **CAL A-F** complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit. If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Calculation of Results

For EIASON® Anti Cardiolipin IgG/IgM kit a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is recommended.

Sample Assay Data

Typical results obtained with EIASON® Anti Cardiolipin Screen IgG/IgM:

No	Position	OD 1	OD 2	Mean	C1	C2	Mean decl.	Conc.	CV %
CALA A 1/B 1		0.050	0.050	0.050	0.1	0.1	0.1	0.0	0
CALB C 1/D 1		0.322	0.300	0.311	7.8	7.0	7.4	7.5	7
CALC E 1/F 1		0.520	0.519	0.520	15.32	15.2	15.2	15.0	0
CALD G 1/H 1		0.803	0.824	0.814	29.2	30.5	29.9	30.0	3
CALE A 2/B 2		1.226	1.191	1.209	61.6	58.2	59.9	60.0	3
CALF C 2/D 2		1.640	1.637	1.638	120.5	119.9	120.2	120.0	0

Expected Values

IgG U/ml	
Normal	< 10
Positive	≥ 10

IgM U/ml	
Normal	< 7
Positive	≥ 7

It's recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-Cardiolipin. The values should be regarded as guidelines only

PERFORMANCE CHARACTERISTICS

Parallelism

Selected sera containing IgG and IgM-antibodies were diluted with sample buffer and assayed in the EIASON® Anti Cardiolipin IgG/IgM kit.

Anti-Cardiolipin	Sample	Dilution	Observed (U/ml)	Expected (U/ml)	O/E
IgG	1	1:200	126.7		
		1:400	63.7	63.4	100%
		1:800	32.9	31.7	104%
		1:1600	14.1	15.8	89%
		1:3200	7.2	7.9	91%
IgG	2	1:100	112.3		
		1:200	56.1	56.2	101%
		1:400	25.0	28.1	89%
		1:800	12.0	14.0	86%
		1:1600	6.0	8.6	86%
IgM	3	1:100	55.0		
		1:200	27.0	27.5	98%
		1:400	13.0	13.8	94%
		1:800	6.4	6.9	93%
IgM	4	1:200	46.5		
		1:400	23.2	23.3	100%
		1:800	10.9	11.6	94%
		1:1600	5.2	5.8	90%
		1:3200	2.8	2.9	97%

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of four samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations each:

IgG			IgG		
Intra-Assay			Inter-Assay		
Sample No	Mean (U/ml)	CV (%)	Sample No	Mean (U/ml)	CV (%)
1	29.1	5.4	1	32.9	3.8
2	62.5	5.8	2	70.9	2.5
3	109.4	4.1	3	118.3	2.7
IgM			IgM		
Intra-Assay			Inter-Assay		
Sample No	Mean (U/ml)	CV (%)	Sample No	Mean (U/ml)	CV (%)
1	8.4	3.7	1	10.3	3.4
2	40.1	4.5	2	47.0	3.3
3	58.6	5.3	3	79.1	2.5

Assay precision and sensitivity

Analytical sensitivity IgG:	1.0 U/ml
Analytical sensitivity IgM:	0.5 U/ml

Specificity

The microplate is coated with highly purified Cardiolipin and human β 2-Glycoprotein I. Special coating processes, developed by the manufacturer guarantee for the native immunogenic structure of Cardiolipin after immobilisation on the solid phase. The Anti-Cardiolipin test kits are specific only for autoantibodies against Cardiolipin or to the complex of Cardiolipin and β 2-Glycoprotein I. No cross reactivity was observed to anti-DNA antibodies and those types of antibodies occurring in Syphilis.

Calibration

The assay system is calibrated against the internationally recognised reference sera from E.N. Harris, Louisville, and the IgG specific reference material IRP 97/656 and HCAL/EY2C9.

LIMITATIONS OF PROCEDURE

The EIASON® Anti Cardiolipin IgG/IgM is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Useful publications

- ◇ Luzzana, C., M. Gerosa, P.Riboldi, and P.L. Meroni. Update on the antiphospholipid syndrome .
J. Nephrol., 2002, 15:342-348.
- ◇ Brandt, J.T., D.A.Triplett,B.Alving, and I.M. Scharrer.Criteria for the diagnosis of lupus anti-coagulants: an update
[Review].Thromb. Haemost., 1995, 74:1185-1190.
- ◇ Khalili, A. and Cooper, R.C. A study of immune responses to Myelin and Cardiolipin in patients with Systemic
Lupus Erythematosus (SLE). Clin. Exp. Immunol., 1991, 85:365-372.
- ◇ Hughes, G.R.V., Harris,E.N. and Gharavi, A.E.The Anticardiolipin Syndrome. J. Rheumatol. 1986; 13, 3: 486 - 489.
- ◇ Molina, J.F., S. Gutierrez-Urena, J. Molina, O.Uribe, S. Richards, C. De Ceulaer, C. Garcia, W.A. Wilson,
A.E.Gharavi, and L.R. Espinoza.Variability of anticardiolipin antibody isotype distribution in 3 geographic
populations of patients with systemic lupus erythematosus. J. Rheumatol., 1997, 24:291-296.
- ◇ Sinico,R.A.,B.Bollini,E.Sabadini,L.Di Toma,and A.Radice.The use of laboratory tests in diagnosis and monitoring of
systemic lupus erythematosus. J. Nephrol., 2002, 6:S20-27.
- ◇ Domke, N. and Siegert, G. Phospholipidantikörper und ihre klinische Bedeutung. Zeitschrift für Klinische Medizin 1988; Heft 16: 1399 – 1401.

Pipetting scheme

1. Dilution	Samples 1:100 with BU	
2. Pipetting	CAL A-F NC PC diluted samples	100 µl
3. Incubation	30 min at room temperature (20-25°C)	
4. Washing	wash 3 x : aspirate or decant add 300 µl WASH aspirate or decant and dry on an absorbent material	
5. Pipetting	CONJM or CONJG	100µl
6. Incubation	15 min at room temperature (20-25°C)	
7. Washing	wash 3 x : see step 4	
8. Pipetting	SUB	100µl
9. Incubation	15 minutes at room temperature (20-25°C)	
10. Pipetting	STOP	100µl
15. Reading	450nm (RF 620nm) Optional Overage Filter: 405nm, Factor: 3 (dependent on photometer), reading within 5 min. Calculation: 4-parameter	

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	IgG U/ml
Normal	< 10
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	IgM U/ml
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Positive	≥ 7
