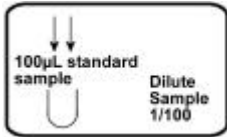
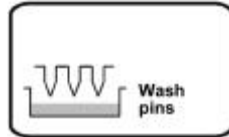


## Test Procedure

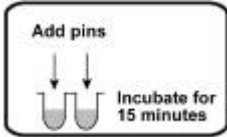
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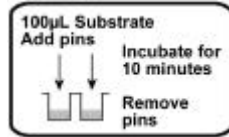
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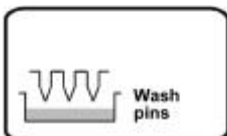
2.



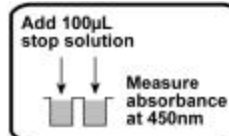
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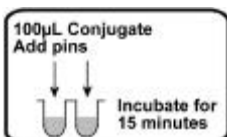
3.



7.



4.



## MELISA™ ACL

Micropin enzyme-linked immunosorbent assay for anti-cardiolipin antibodies

**Combined ACL IgG / IgM**  
Combined anti-cardiolipin antibodies  
IgG / IgM assay kit  
**Code: M4796A**

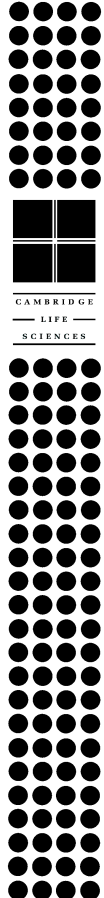
**ACL IgG**  
Anti-cardiolipin antibodies IgG assay kit  
**Code: M4596A**

**ACL IgM**  
Anti-cardiolipin antibodies IgM assay kit  
**Code: M4696A**

Instructions for Use

Cambridge Life Sciences  
14 St. Thomas' Place  
Cambridgeshire Business Park  
Ely, Cambs. CB7 4EX. UK  
Tel: 01353 645200 Fax: 01353 645250  
e-mail: sales@clsdiagnostics.com  
www.cambridgelifesciences.co.uk

A5360.5  
June '04



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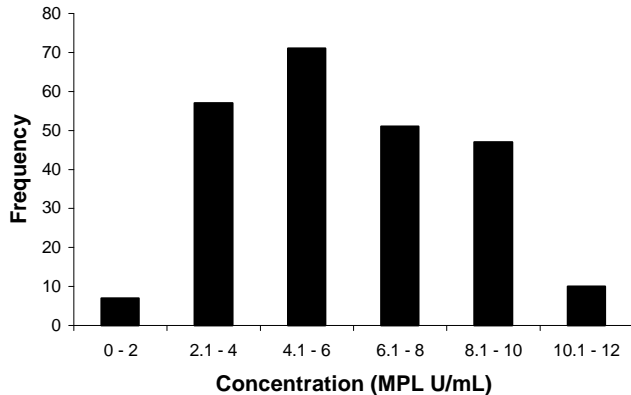
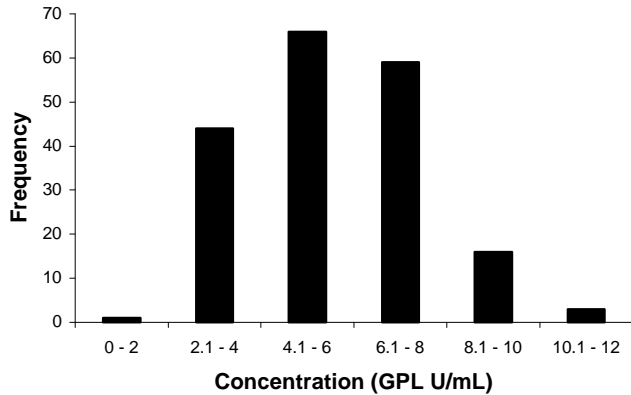
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## 1. Intended Use

The Combined Anti-Cardiolipin (ACL) IgG / IgM, ACL IgG and ACL IgM MELISA™ ACL products are sandwich enzyme immunoassays for the quantitative detection of IgG or IgM classes of antibodies to cardiolipin in human serum.

The ACL IgG and ACL IgM standards are calibrated against the original Rayne Institute Reference Standards supplied by Dr E N Harris now at the Anti-phospholipid Standardisation Laboratory, Division of Rheumatology, University of Louisville, Kentucky 40292, USA.

## 2. Background

Anti-cardiolipin (ACL) antibodies have been strongly associated with venous and arterial thrombosis particularly in recurrent unexplained thrombocytopenia, recurrent foetal loss, myocardial infarction and recurrent stroke.

Elevated levels are found frequently in these patient groups and in patients with acquired immune deficiency syndrome (AIDS).

### 3. Principle

MELISA™ ACL products employ a unique antigen-coated micropin technology, which is ideal for the batch-screening of large numbers of samples for anti-cardiolipin antibodies. The methods utilise a non-competitive sandwich enzyme immunoassay system.

#### First Incubation

MELISA™ ACL micropins are provided coated with purified antigen (cardiolipin and the cofactor  $\beta_2$  Glycoprotein-1). When the micropins are immersed into wells containing standards or diluted sera, any ACL antibodies present will bind to the micropin surface. The micropins are then removed and washed in wash buffer.

#### Second Incubation

The micropins are then dipped into wells containing antihuman-IgG-horseradish peroxidase (HRP) conjugate or antihuman-IgM-HRP conjugate, which will bind to any captured antibodies. Unbound conjugate is removed by washing in wash buffer.

#### Third Incubation

The micropins are then dipped into wells containing a colourless substrate. The intensity of the blue colour formed is proportional to the concentration of ACL antibody bound in the first incubation. The reaction is stopped with the supplied stop solution which changes the blue colouration to yellow.

### 2. Minimum detectable concentration

The minimum detectable concentration, defined as the concentration equal to 2 standard deviations from the mean of the sample diluent, was found to be:

0.24 GPL U/mL (ACL IgG)  
0.14 MPL U/mL (ACL IgM)

### 3. Reference Ranges

MELISA™ ACL was used to determine the ACL antibody levels of 100 plus serum samples measured in duplicate from normal blood donors with no apparent abnormalities. The data was evaluated and the following ranges obtained:

	ACL IgG (GPL U/mL)	ACL IgM (MPLU/mL)
Normal range	< 9.4	< 10.7
Borderline	9.4 - 11.2	10.7 - 13.1
Positive	> 11.2	> 13.1

It is advised that each laboratory establishes its own reference range.

### 13. Performance Characteristics

#### 1. Precision data

##### ACL IgG:

Intra-assay (n=20)	Mean (GPL U/mL)	CV (%)
Sample 1	4.8	6.7
Sample 2	22.2	8.2

##### ACL IgG:

Inter-assay (n=20)	Mean (GPL U/mL)	CV (%)
Sample 1	4.1	16.4
Sample 2	22.3	14.4

##### ACL IgM:

Intra-assay (n=20)	Mean (MPL U/mL)	CV (%)
Sample 1	2.9	8.4
Sample 2	21.9	5.9

##### ACL IgM:

Inter-assay (n=20)	Mean (MPL U/mL)	CV (%)
Sample 1	3.8	15.7
Sample 2	22.0	10.2

### 4. Kit Contents

Standard	ACL IgG GPL U/mL	ACL IgM MPL U/mL
1	0	0
2	6.3	3.8
3	12.5	7.5
4	25	15
5	50	30
6	100	60

The six (6) standards\* are 1.0mL ready to use.

(\* For the Combined MELISA™ ACL IgG / IgM, product code:M4796A, 2 x 6 standards are included i.e. 1 set ACL IgG and 1 set ACL IgM. In addition, 2 x 1 conjugate anti-IgG-HRP and anti-IgM-HRP are used).

- 1 vial wash buffer concentrate (x15), 20mL
  - 1 vial sample diluent, 100mL
  - 1 vial conjugate (anti-Ig(G or M)-HRP), 12mL
  - 1 vial substrate (TMB#), 12mL
  - 1 vial stop solution, 12mL
  - 1 vial Negative control, 1mL ready to use
  - 1 vial Positive control, 1mL ready to use (IgG or IgM positive control or both for the M4796A kit)
  - 1 foil sachet containing 1 set of 96 antigen-coated micropins and 1 flat-well plate
  - 2 round-well plates
  - 2 wash trays
  - 1 instruction leaflet
  - 1 Certificate of Analysis
- # TMB = 3,3', 5,5'-tetramethyl benzidine.

## 5. Storage

The kit should be stored at 2 - 8°C.

Do not use the reagents beyond their expiry date.

Do not freeze.

Keep all reagents away from direct sunlight.

## 6. Safety Precautions

For *in-vitro* diagnostic use only.

For professional use only.

The standards and controls contain human source material. Although found negative when tested for HIV-1 and HIV-2 antibodies, HCV and hepatitis B surface antigen, no test can guarantee their absence. Therefore, the standards and controls should be handled using the same safety precautions employed when handling any potentially infectious material.

Used standards, samples and controls, pipette tips, micropins and plates should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves are worn when handling such items.

Safety data sheets are available on request.

11. Measure the absorbance at 450nm on a 96-well microtitre plate reader.

## 11. Calculation of Results

For each assay, prepare a calibration curve by plotting mean absorbance against standard concentration on linear graph paper, and interpolate unknowns. Alternatively, use a computerised curve-fit program. Any sample giving values above the standard range should be further diluted and retested.

## 12. Quality Control

Good laboratory practice requires that quality control samples be included in every run to check on assay performance.

Positive and Negative controls are provided in the kit.

These controls are ready-to-use and do not require dilution. If either control value falls outside the quoted range, the results are invalid and the assay should be repeated.

repeat in the second wash tray. Avoid the wash buffer making contact with the micropins top. If this occurs, remove the micropins, flick off the excess wash buffer and repeat wash step. After washing, flick off excess wash buffer ensuring that the micropins are free of wash buffer and bubbles.

7. Transfer the micropins to the conjugate plate and gently tap the micropins to ensure the micropins are evenly covered with solution, and are correctly located in the wells. Incubate for 15 minutes at room temperature (18 - 25°C). During the incubation period, prepare the substrate plate by dispensing 100µL of **substrate** into each well of the flat-well plate. Also, prepare for the final micropin washing stage by filling the wash trays with 50mL fresh wash buffer per wash tray.
8. After incubation, wash the micropins as in step 6.
9. Transfer the micropins to the substrate plate and gently tap the micropins to ensure the micropins are evenly covered with solution, and are correctly located in the wells. Incubate for 10 minutes at room temperature (18 - 25°C).
10. After the 10-minute incubation in the substrate solution, carefully remove the micropins from the plate and discard them. Gently tap the plate until the blue colour is evenly distributed within the well and then add 100µL of **stop solution** to each well. The developed colour will change from blue to yellow.

## 7. Sample Handling

The assay may be performed on human serum samples.

Repeated freeze-thawing is not advisable.

Do not heat treat samples.

## 8. Additional Reagents and Equipment

Deionised water.

Precision micropipettes and disposable tips to deliver 5 - 1000µL.

Multichannel micropipette or repeating dispenser to deliver 100µL.

500mL and 50mL measuring cylinder for reagent preparation.

96-well microplate reader with 450nm filter.

Software package (optional).

## 9. Procedural Precautions

Carefully read instructions for use before starting with the assay.

Allow all reagents to equilibrate to room temperature (18 - 25°C) before use for a minimum of two hours.

Avoid the use of icteric, lipaemic or grossly haemolysed samples.

Always change pipette tips between different standards, samples or controls to prevent sample carryover.

Never allow the same pipette tip to be used with different reagents. Special care is needed to prevent contamination of the substrate by the conjugate.

The substrate should be colourless. Any colouration indicates substrate contamination and the substrate should be discarded.

The micropin washing procedure is critical for the successful performance of the test, especially between conjugate and substrate incubations (i.e. the second and third incubations).

Do not use the kit beyond the expiry date given on the box.

Reagents must not be re-used.

## 10. Assay Procedure

1. Prepare the wash buffer as follows: dilute contents of the **wash buffer concentrate** (x15) vial to 300mL with deionised water.
2. Dilute patient samples 1/100 using the **sample diluent** e.g. 10µL sample added to 990µL diluent. The **standards and controls** do not require dilution.
3. Prepare the sample plate by dispensing 100µL of each **standard, control** or diluted patient sample into appropriate wells of a round-well plate. It is recommended that samples be tested in duplicate.
4. Prepare for the micropin washing stage by pouring 50mL wash buffer into each of the two wash trays, so that it fills the central reservoir. This should be measured accurately. Label as wash tray 1 and 2.
5. Remove the **antigen-coated micropins** from the foil sachet. Transfer the micropins to the sample plate and gently tap the micropins to ensure the micropins are evenly covered with solution, and are correctly located in the wells. Incubate for 15 minutes at room temperature (18 - 25°C). During the incubation period, prepare the conjugate plate by dispensing 100µL **conjugate** into each well of the second round-well plate.
6. After incubation, transfer the micropins to the first wash tray and gently agitate by sideways and up and down movements for at least 10 seconds. Flick off the excess wash buffer or gently drain on absorbent paper and