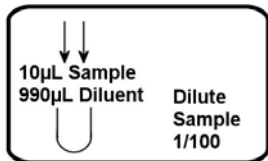
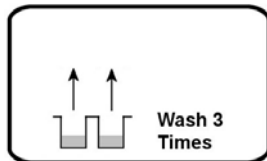


## Test Procedure

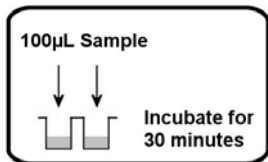
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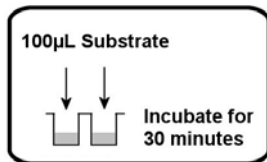
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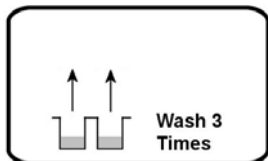
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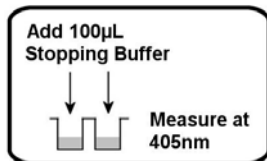
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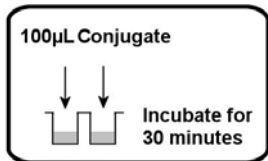
3.



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A5261.14  
May '05



## AUTOZYME™ ACL

ACL IgA Z4496

ACL IgG Z4596

ACL IgM Z4696

Instructions for Use



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## Kit Contents Symbols

<b>CAL</b>	Calibrators
<b>CONTROL -</b>	Negative Control
<b>CONTROL +</b>	Positive Control
<b>BUF WASH</b>	Wash Buffer
<b>DIL SPE</b>	Sample Diluent
<b>CONJ</b>	Conjugate solution
<b>SUB</b>	Substrate solution
<b>STOP</b>	Stop Solution
<b>SORB</b>	Solid Phase – Antigen Coated Wells

## 1. Intended Use

The AUTOZYME™ ACL anti-cardiolipin (ACL) IgA, IgG and IgM are sandwich immunoassays for the quantitative detection of anti-cardiolipin antibodies of IgA, IgG and IgM classes in human serum. The AUTOZYME™ ACL IgA, IgG and IgM results are expressed in APL U/mL, GPL U/mL and MPL U/mL respectively. AUTOZYME™ ACL has been specifically designed with automation in mind and can be adapted to automated immunoassay systems.

## 2. Background

Anti-cardiolipin antibodies have been strongly associated with venous and arterial thrombosis particularly in recurrent unexplained thrombocytopenia, recurrent foetal loss, myocardial infarction and recurrent stroke. Recent studies indicate that elevated levels of ACL IgA as well as ACL IgG and IgM are found frequently in these patient groups.

## 3. Principle

The AUTOZYME™ ACL employs a unique antigen-coated microwell technology, which is ideal for the batch-screening of large and small numbers of samples for ACL. The method utilises a non-competitive sandwich enzyme immunoassay system.

### First incubation:

AUTOZYME™ ACL wells are provided coated with purified antigen (cardiolipin and  $\beta_2$ -glycoprotein 1 cofactor). When calibrators or diluted sera are added, any ACL present will bind to the well surface. The wells are then washed in wash buffer.

### Second incubation:

Goat anti-human IgA, IgG or IgM peroxidase conjugate is added to the wells, which will bind to any captured ACL. Unbound conjugate is removed by washing in wash buffer.

## Bibliography

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Hughes, G.R.V. *et al*, (1989). Anti-phospholipid syndrome linking many specialities, *Ann. Rheum. Diseases* **45**, 355-356.

Unander, A.M. *et al*, (1987) Anti-cardiolipin antibodies and complement in ninety-nine women with habitual abortion. *Am. J. Obstet. Gynecol.* **156/1**, 114-119.

### 13. Safety Precautions

For *in vitro* diagnostic use only.

For Professional Use only.

The **substrate** contains ABTS™ which is harmful if swallowed in copious amounts and may cause skin irritation if exposed for prolonged periods. In case of skin contact, wash with soap and water. Flush eyes with copious amounts of water.

The **calibrators 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 calibrators should be handled using the same safety precautions employed when handling any potentially infectious material.

Used calibrators, controls, samples, pipette tips 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.

ABTS™ (2, 2'-azino-bis (3-ethylbenzothiazoline-6 sulphonic) acid) is a trademark of Roche Diagnostics.

### Third incubation:

A pale green substrate is then added to the wells. The intensity of the green colour formed is proportional to the concentration of ACL bound in the first incubation. The reaction is stopped with a low pH solution.

### 4. Kit Contents

6 vials calibrators (ready-to-use) (6 ACL IgA or 6 ACL IgG or 6 ACL IgM), 1.5 mL each:

Calibrator	ACL IgA (APL U/mL)	ACL IgG (GPL U/mL)	ACL IgM (MPL U/mL)
1	0.0	0.0	0.0
2	6.3	6.3	3.8
3	12.5	12.5	7.5
4	25.0	25.0	15.0
5	50.0	50.0	30.0
6	100.0	100.0	60.0

1 vial wash buffer concentrate (PBS), 67 mL

1 vial sample diluent (BSA/PBS), 100 mL

1 vial conjugate:

(anti-IgA HRP or anti-IgG HRP or anti-IgM HRP), 15 mL

1 vial substrate (ABTS), 15 mL

1 vial stopping buffer (oxalic acid), 15 mL

1 foil sachet, containing 1 set of antigen-coated microwells

1 vial Positive control, 1.5 mL (ready-to-use)

1 vial Negative control, 1.5 mL (ready-to-use)

1 instruction leaflet

1 QC certificate

### 5. Storage

The kit should be stored refrigerated at 2-8°C. Do not use the reagents beyond their expiry date. Do not freeze. Keep all reagents away from direct sunlight.

## 6. Sample Handling

AUTOZYME™ ACL must be performed with human serum samples.

Samples should be assayed within 24 hours of collection or stored frozen at -15°C or colder. Repeated freeze-thawing is not advisable.

Do not heat treat samples prior to assay.

## 7. Additional Reagents and Equipment Required

Deionised or freshly distilled water.

Precision micropipettes to deliver 10 - 1000 µL.

Multichannel micropipette or repeating dispenser to deliver 50 µL and 100 µL.

1000 mL measuring cylinders for reagent preparation.

Automated plate washer (optional).

96-well microplate reader with 405 nm filter.

Software package (optional).

## 8. Procedural Precautions

Allow all reagents to equilibrate to room temperature (18°C to 25°C) before use for a minimum of 2 hours.

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

Always change tips between different calibrators, samples or control sera to prevent sample carryover.

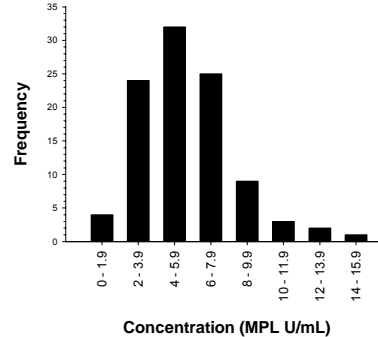
Never allow the same pipette tip to be used with different reagents.

Special care is needed to prevent contamination of the substrate by the conjugate.

The substrate should be pale green. Any green colouration above 0.200 indicates substrate contamination and should be discarded.

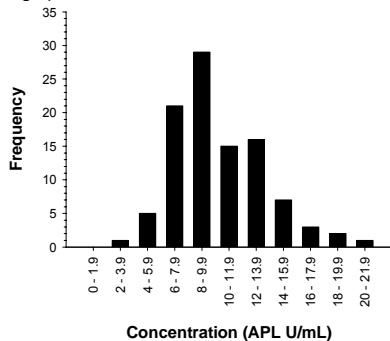
The well washing procedure is critical for the successful performance of the test, especially between conjugate and substrate incubations (i.e. the second and third incubations).

	MPL U/mL
Normal range	≤ 9.8
Weak positive	9.9-13.2
Moderate positive	13.3-50.0
High positive	≥ 50.1

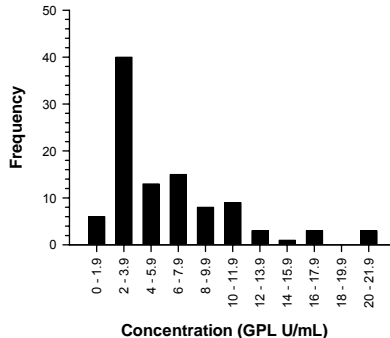


It is recommended that each laboratory determines its own reference range.

Normal range  $\leq 10.2$   
 Weak positive 10.3 - 14.3  
 Moderate positive 14.4 - 80.0  
 High positive  $\geq 80.1$



Normal range  $\leq 13.3$   
 Weak positive 13.4 - 19.9  
 Moderate positive 20.0 - 80.0  
 High positive  $\geq 80.1$



AUTOZYME™ ACL has been designed so that AUTOZYME™ ACL IgA and ACL IgG and ACL IgM can be run simultaneously on the same ACL plate if required. All reagents are common with the exception of calibrators, controls and conjugates.

Do not use the kit beyond the expiry date given on the label. Unused reagents are stable at 2 - 8°C for 1 month after first opening the container. However, multiple re-use could increase the risk of reagent contamination.

## 9. Assay Procedure

- Prepare the following reagents:  
**Wash buffer:** dilute contents of wash buffer concentrate vial to 1000 mL (1/15) with deionised water or proportionally less if not using the whole kit.
- Dilute the patient samples by 1/100 using the sample diluent e.g. 10  $\mu$ L sample added to 990  $\mu$ L diluent.  
 The **Calibrators and kit Controls** do not require dilution.
- Remove the antigen-coated microwells from the foil sachet and seal any unrequired wells in the resealable pouch, along with the desiccant sachet.
- Dispense 100  $\mu$ L of each calibrator or diluted patient sample into appropriate wells. Incubate for 30 minutes at room temperature (18 - 25°C). It is recommended that samples be tested in duplicate.
- Gripping the frame on the long sides to retain the strips, flick out the contents of the wells. Using the diluted wash buffer, wash the wells at least three times either with an automated plate washer set to at least 300  $\mu$ L per well, or by adding 300  $\mu$ L to each well and flicking out, gripping the frame on the long sides to retain the strips. Alternatively use a wash bottle. Blot the wells on absorbent material to remove any residual liquid.
- Add 100  $\mu$ L conjugate to each well and incubate for 30 minutes at room temperature (18 - 25°C).

7. Gripping the frame on the long sides to retain the strips, flick out the contents of the wells. Wash the wells at least three times using the same procedure as in step 5.
8. Dispense 100 µL substrate into each well, ensuring that it is initially pale green and incubate for 30 minutes at room temperature (18 - 25°C).
9. Stop the reaction by adding 100 µL of stopping buffer.
10. Measure the absorbance at 405 nm on a 96-well microplate reader.

## 10. Calculation of Results

For each assay, prepare a calibration curve by plotting mean absorbance against calibrator concentration on linear graph paper, and interpolate unknowns. Alternatively, use a computerised curve-fit program.

Any sample giving values above the calibrator range should be diluted and retested.

## 11. Quality Control

Good laboratory practice requires that quality control specimens be included in every run to check on assay performance. The kit control ranges are provided on the certificate of analysis. If either control value falls outside the quoted range, the results are invalid and the assay should be repeated.

The ACL IgG and ACL IgM calibrators 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. AUTOZYME™ ACL IgG is calibrated to reference material 97/656.

## 12. Performance Characteristics

### 1. Precision data:

	APL		GPL		MPL	
	U/mL	CV %	U/mL	CV %	U/mL	CV %
Intra-assay (n=20)						
Sample 1	14.1	3.5	25.9	9.0	7.3	7.8
Sample 2	38.9	5.3	63.9	7.3	26.2	11.3
Sample 3	69.9	10.1	71.2	8.5	52.8	13.1
Inter-assay (n=3)						
Sample 1	12.6	10.7	22.9	13.3	6.6	12.3
Sample 2	36.8	5.8	60.7	4.6	25.6	2.2
Sample 3	69.3	6.0	73.3	5.7	56.9	6.4

### 2. Minimum detectable concentration:

The minimum detectable concentration, defined as the concentration equal to 2 standard deviations from the mean of 20 replicate determinations of the sample diluent, was found to be:

ACL IgA:	3.0 APL U/mL
ACL IgG:	0.3 GPL U/mL
ACL IgM:	0.6 MPL U/mL

### 3. Reference values:

AUTOZYME™ ACL was used to determine the ACL IgA, IgG and IgM levels of 204 serum samples from normal blood donors with no apparent abnormalities. The ranges are as follows: