



AUTOZYME™ ACL

- REF Z4496: ACL IgA kit**
- REF Z4596: ACL IgG kit**
- REF Z4696: ACL IgM kit**

Instructions for Use

IVD For in vitro diagnostics use only

96 Tests

Store at 2 - 8°C



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A5261.17
Jun '11

Kit Contents Symbols

- CONTROL -** Negative Control
- CONTROL +** Positive Control
- BUF WASH x15** Wash Buffer x15 concentrate
- DIL SPE** Sample Diluent
- CONJ** Conjugate solution
- SUB** Substrate solution
- STOP** Stop Solution
- SORB** 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 result 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 β_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.

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.

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.

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

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

1 vial wash buffer concentrate (PBS), 67 mL
1 vial sample diluent (FC/S/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

Second incubation:
Coat 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.

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.

Test Procedure

- 1.**
- 2.**
- 3.**
- 4.**
- 5.**
- 6.**
- 7.**

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 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).

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 interchange kit components from different lots.

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 µL sample added to 990 µL 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 µL 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.

12b Precision data:

Intra-assay (n=20)	APL U/mL	CV	GPL	CV	MPL	CV
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	APL U/mL	CV	GPL	CV	MPL	CV
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

12c Reference values:

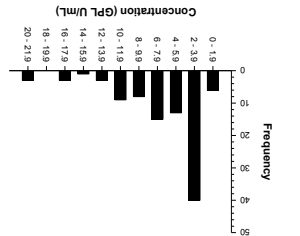
AUTOTZYME™ ACL was used to determine the ACL IGA, IgG and IGM levels of 204 serum samples from normal blood donors with no apparent abnormalities.

The ranges are as follows:

APL U/mL	Weak positive	Moderate positive	High positive
≤ 10.2	10.3 - 14.3	14.4 - 80.0	≥ 80.1

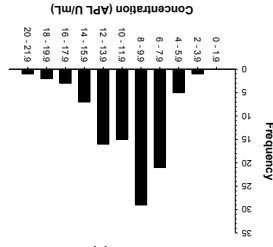
GPL U/mL

Normal range	Weak positive	Moderate positive	High positive
≤ 13.3	13.4 - 19.9	20.0 - 80.0	≥ 80.1



MPL U/mL

Normal range	Weak positive	Moderate positive	High positive
≤ 9.8	9.9-13.2	13.3-50.0	≥ 50.1



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.

Bibliography

Asherson, R.A. *et al.* (1987) Recurrent stroke and multi-infarct dementia in systemic lupus erythematosus: association with anti-phospholipid antibodies. *Ann. Rheum. Diseases*, **46**, 605-611.

Gharavi, A.E. *et al.* (1987) Anti-cardiolipin antibodies - isotype distribution and phospholipid specificity. *Ann. Rheum. Diseases*, **46**, 1-6.

Harris, E.N. *et al.* (1983) Anti-cardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet*, 1211-1214.

Harris, E.N. *et al.* (1985) Anti-phospholipid antibodies. *Clinics in Rheumatic Diseases*, 11/3, 591-609.

Valentin, G. *et al.* (1992) A new player in the anti-phospholipid syndrome: the β2-Glycoprotein-1 co-factor. *Autoimmunity*, **14**, 105-110.

Loizou, S. *et al.* (1985) Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): standardisation and quantitation of results. *Clin. Exp. Immunol.*, **62**, 738-745.

Hughes, G.R.V. *et al.* (1989). Anti-phospholipid syndrome linking many specialties. *Ann. Rheum. Diseases* **48**, 355-356.

Ulander, 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.