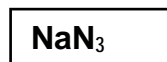
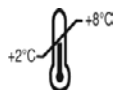




IgM CLASS
ANTI-CARDIOLIPIN ANTIBODY
96 WELL ELISA KIT

PACKAGE INSERT
INSTRUCTIONS AND INFORMATION

Store at 2-8°C



Product Code: IGMACA
Document Number KBK-0002
Issue No: 13

1. INTENDED USE

This kit is an *in-vitro* laboratory test for the quantitation of IgM class Anti-Cardiolipin Antibodies (ACA) as an aid in the diagnosis of “antiphospholipid syndrome” (APS) which may be associated with venous and/or arterial thrombosis, recurrent foetal loss and thrombocytopenia.

2. INTRODUCTION

The kit contains one 96-microwell plate which is divided into 12 strips of 8 wells each and is vacuum-sealed in a re-sealable foil bag. Each well can be separated from the module (breakaway) allowing a total of 42 patient samples, plus standards and controls in duplicate to be assayed.

The quantitation of IgM Anti-Cardiolipin Antibodies is determined by adding a diluted sample of patient serum to cardiolipin-coated microwells. If Anti-Cardiolipin Antibodies are present in the serum, they will bind to the cardiolipin bound to the solid phase.

The kit is supplied with four standards plus positive and negative control sera. The standards have been calibrated to the internationally accepted standards (1). Scientists, previously associated with the development of the kit, were instrumental in the identification of the serum co-factor later confirmed to be β 2-glycoprotein 1, and this co-factor is incorporated in the kit (2).

To ensure accurate and reproducible results, standards, controls and test sera are treated in the same manner and assayed in parallel each time. Test sample quantitation is thus determined by the intensity of colour relative to that of the standards supplied.

The presence of Anti-Cardiolipin Antibodies has been associated with the antiphospholipid syndrome (3), which is characterised by the following clinical conditions:

- Venous thrombosis (4)(5)
- Arterial thrombosis (6)(7)
- Recurrent foetal loss (8)(9)(10)
- Thrombocytopenia (11)

It has also been found that perhaps as many as 50% of SLE patients have Anti-Cardiolipin Antibodies. (12)(13)(14)

It is recommended that the entire instruction booklet be reviewed prior to testing.


3. PRINCIPLE OF THE TEST

The anti-cardiolipin antibody test is an indirect solid-phase enzyme immunoassay that detects cardiolipin specific IgM in human serum.

Cardiolipin (coated onto microwells) is reacted with specific IgM in patient's serum, forming a cardiolipin/anti-cardiolipin IgM complex. Anti-human IgM conjugated with the enzyme horseradish peroxidase (HRP) binds to immobilized IgM.

This complex is then reacted with a specific substrate (ABTS) to yield a green colour. Following termination of colour development, the absorbance, measured at 405 or 410nm, is related to the level of cardiolipin-specific serum IgM.

4. KIT COMPONENTS

1. **MP** CARDIOLIPIN COATED MICROWELL PLATE (Red) - containing 12 strips, each strip consisting of 8 break-away wells coated with cardiolipin. The plate is vacuum sealed.
2. **REF** STANDARD A (red cap) 64 units/mL, 200µL
3. **REF** STANDARD B (red cap) 32 units/mL, 200µL
4. **REF** STANDARD C (red cap) 16 units/mL, 200µL
5. **REF** STANDARD D (red cap) 2 units/mL, 200µL
6. **CONTROL +** POSITIVE CONTROL (red cap) Units on vial label, 200µL
7. **CONTROL -** NEGATIVE CONTROL (red cap) < 2 units/mL, 500µL
8. **SB** DILUENT (amber cap) - 0.15M phosphate buffered saline (pH 7.2), containing β2-glycoprotein 1, 52mL. Ready for use.
9. **WASH 20x** WASH BUFFER CONCENTRATE (white cap) - 20x concentrated 0.15M phosphate buffered saline (pH 7.2), 52mL. To be diluted 1/20 prior to use.
10. **CONJ** CONJUGATE (red cap) - Anti-human IgM horseradish peroxidase (HRP) conjugate, 75µL. To be diluted 1/500 prior to use.
11. **SUB** SUBSTRATE (green cap)- 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid 0.55mg/mL hydrogen peroxide 30% w/v at 0.002% in 0.1M citrate phosphate buffer (pH 4.3), 15mL. Ready for use.
12. **STOP** STOP SOLUTION (clear cap) - 2.5% sodium fluoride, 15mL. Ready for use.
13.  PACKAGE INSERT

5. OTHER EQUIPMENT REQUIRED BUT NOT SUPPLIED

This protocol has been validated for the Triturus™ Analyser

1. MICROWELL PLATE READER (capable of reading at 405 or 410nm)
2. WASH BOTTLE - 500mL or suitable MICROWELL PLATE WASHER
3. 25mL AND 500mL MEASURING CYLINDERS
4. MULTICHANNEL PIPETTE (50µL)
5. REAGENT TROUGHES
6. VARIABLE VOLUME PIPETTORS (10µL – 5mL) AND DISPOSABLE TIPS
7. PLATE LID
8. 96 WELL DILUTION PLATES OR TUBES

6. ASSAY PROCEDURE

- Review all instructions thoroughly before testing.
 - Room temperature incubations should be performed at 20 - 24°C.
 - The kit components should not be left at room temperature for longer than the procedure requires. Store kits and kit components at 2 - 8°C when not in use.
 - Briefly centrifuge or tap vials containing standards, controls and conjugate to ensure that the contents are drawn to the bottom of the tube. Mix contents by pipetting up and down prior to use.
 - The WASH BUFFER CONCENTRATE (20x) may crystallise at 4°C. Incubate in a 37°C water bath until crystals have dissolved.
 - To ensure accurate quantitation, it is essential that all pipettes used in the assay are calibrated and a fresh tip used for standard, control and samples.
 - Once started, finish the assay without interruption.
1. Perform a 1/20 dilution on each of the standards, negative control and positive control and the test samples (10µL of sample to 190µL of diluent). Mix well. It is recommended that the Standards, Controls and Samples be run in duplicate.
 2. Remove the required number of cardiolipin coated microwell strips from the sealed bag and place them in the holder. Ensure unused microwells are resealed and stored at 4°C.
 3. Pipette 50µL to the corresponding cardiolipin-coated test wells. To avoid cross-contamination, use a new pipette tip for each test sample.
 4. Cover the microwells with a lid, and incubate for 30 MINUTES ± 5 MINUTES AT ROOM TEMPERATURE.
 5. Dilute the WASH BUFFER CONCENTRATE (20x) 1/20 with distilled water. Place in either a wash bottle or plate washer reservoir.
 6. For MANUAL washing: Discard or aspirate the samples from all wells and fill all wells with diluted WASH BUFFER and discard immediately. Refill wells with WASH BUFFER. Leave for **2 minutes** then discard. Refill wells again with WASH BUFFER. Leave for **2 minutes** then discard and blot dry all excess moisture by tapping the plate on absorbent paper.

For AUTOMATED washing: Aspirate the samples from the wells and then fill all wells with diluted WASH BUFFER (350µL/well) soak for 7 seconds. Repeat the above for 4 cycles.
NB: The washing protocol needs to be programmed for a STRIP WASH not a plate wash.
 7. Dilute the anti-human IgM HRP CONJUGATE 1/500 in DILUENT and mix gently. Pipette 50µL/well of the freshly diluted anti-human IgM HRP CONJUGATE to the microwells.
 8. Replace the lid and incubate the plate for 45 MINUTES ± 5 MINUTES AT ROOM TEMPERATURE.
 9. Wash the plate as in step 6, but do not include a soak time.
 10. Pipette 50µL/well of SUBSTRATE to the microwells. Incubate plate at ROOM TEMPERATURE for 15 minutes ± 2 minutes.
 11. Stop the reaction by adding 50µL/well of STOP SOLUTION to the microwells.
 12. Read the plate at 405nm or 410nm.

- Calculate the corrected mean optical densities (O.D.) by subtracting the mean O.D. of the Negative Control from the mean O.D. of all standards and samples.

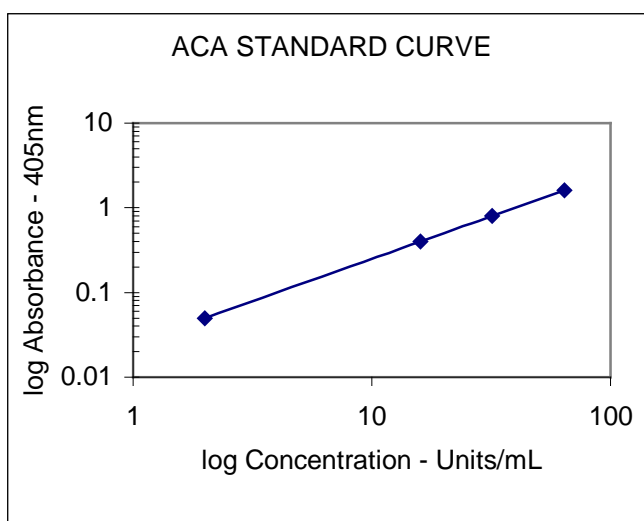
The corrected optical density of Standard A should be greater than 1.3 and less than 2.0. Inaccurate results may be obtained if this condition is not met.

- Plot the corrected mean optical density values of Standard A, Standard B, Standard C and Standard D against the concentration. Construct a standard curve using the line of best fit. Linear regression or 4PL. Determine the controls and test sample values from the curve.

NOTE: A new standard curve must be constructed for each assay.

Samples giving values above 64 units/mL may be diluted 1/5 or 1/10 in NEGATIVE CONTROL and re-assayed.

An example of a typical standard curve (log-log using best fit curve).



7. INTERPRETATION OF RESULTS

It is recommended that all laboratories independently establish their own normal range. As a guide however, an independent study obtained the following results from randomly selected blood donors.

IgM ACA	SERUM (n=192)	CITRATED PLASMA (n=499)
95th Percentile value	4.44units/mL	3.33 units/mL

The units/mL described are approximately equivalent to the MPL units/mL described by Harris et al. (1). Additionally, the IgM standards have been measured against an affinity-purified IgM Anti-Cardiolipin Antibody and one IgM unit/mL was found to be equivalent to one $\mu\text{g/mL}$. (15).

8. PRECAUTIONS

- Do not substitute PBS wash solutions that contain Tween-20 or other detergents for the one supplied with the kit. Also, if a microwell plate washer is used, please ensure that if it has been used previously with a Tween-20 containing wash solution that it has been thoroughly rinsed clean to remove all traces of Tween-20.

2. All standards and controls contain human serum, which has been tested and found negative for HBsAg and antibodies to HIV. However they should still be treated as though they were potentially infectious.
3. Do not substitute any component for the ones supplied with the kit.
4. All reagents contain thiomersal as a preservative except the wash, substrate and stop solutions. Caution must be exercised in handling/disposing of these products.
5. Heat inactivation or repeated freezing and thawing of serum or citrated plasma samples may cause erroneous results.
6. Performing the assay at temperatures other than those stated may cause erroneous results.
7. The performance of the assay has not been evaluated using microplate readers in the dual wave length mode.
8. Care should be taken to reduce microbial contamination of reusable kit components.
9. Do not use kit components beyond the expiry date, and do not mix reagents from different kits.
10. It is recommended that "in-house" controls be included with each assay.
11. Some kit components contain sodium azide, which may react with lead or copper pipes to form highly explosive compounds (flush with copious amounts of water to prevent build up).

9. REAGENT STORAGE AND SHELF LIFE

- All kit components must be stored at 2 - 8⁰C. All reagents are stable until labelled expiration date when stored at 2 - 8⁰C.
- If additional kit components are required contact your distributor.

10. SPECIMEN COLLECTION

- Blood should be collected by venepuncture.
- Specimens should be frozen until required for use

11. QUALITY CONTROL

- The Positive Control and Negative Control should be run each time the assay is performed.
- If the Positive Control fails to read within the specified range or the Standard A absorbance is outside the limits as described in 6.13, the assay should be repeated.
- The absorbance of Standard D should be greater than the Negative Control.

12. LIMITATIONS

- As with other diagnostic test procedures, the results obtained with the ACA Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in itself.
- Sera from patients with other autoimmune diseases may contain autoantibodies. Patients with syphilis or other infections may have positive results.
- It is recommended that the test be performed on serum or citrated plasma. Use of whole blood or other samples has not been established.

- Haemolysed, lipemic or contaminated specimens should not be used.

13. TECHNICAL PERFORMANCE

A. INTRA-ASSAY VARIATION

The intra-assay coefficient of variation (CV) was shown by assaying 2 samples in duplicate.

Sample No.	n	Mean (units/mL)	SD	%CV
1	8	13.1	0.77	4.9
2	8	55.3	2.60	3.5

B. INTER-ASSAY VARIATION

The inter-assay coefficient of variation (CV) was shown by repeatedly assaying 2 positive samples using 3 different Lots of kits.

Sample No.	n	Mean (units/mL)	SD	%CV
3	5	6.74	0.56	8.4
4	5	33.2	2.77	8.4

14. REFERENCES

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15. LIMITED EXPRESS LIABILITY

The manufacturer makes no express warranty other than the diagnostic kit will measure IgM anti-cardiolipin antibodies when used in accordance with the manufacturer's instructions. The use of the diagnostic kit for any other purpose or for the clinical diagnosis of a disease state is outside the intended use of this product.

The manufacturer disclaims any and all implied merchantability, fitness for use or implied utility for any other purpose. Any or all damages for failure of the diagnostic kit to perform according to its instructions are limited to the replacement value of the kit.

In some jurisdictions the law makes these disclaimers unenforceable and, accordingly all or part of the disclaimer may not apply to all users.



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