ZYMUTEST ACA-APA
IgA - Isotype
Ref RK029C (96 tests)

ELISA method for quantitative determination of Anti-Cardiolipin / Anti-Phospholipid antibodies

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE:

The ZYMUTEST ACA-APA, IgA ELISA kit, is an optimized enzyme immuno-assay designed for measuring anti-cardiolipin / anti-phospholipid antibodies of the IgM isotype, in human plasma or serum, or in any biological fluid where these antibodies must be measured.
This kit is for research use only and should not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

The ZYMUTEST ACA-APA, IgA Kit, specifically measures human anti-cardiolipin / anti-phospholipid antibodies of the IgA isotype, reactive with immobilised and saturated cardiolipin. IgG or IgM isotypes are not measured. These isotypes can be assayed with ZYMUTEST ACA-APA IgG or ZYMUTEST ACA-APA IgM.
This optimised assay is designed with highly reactive cardiolipin, which has a well-controlled presentation, stabilised, and saturated. This reliable method then provides high reproducibility, high sensitivity and high specificity, and offers an optimised discrimination between normal individuals and pathologies with presence of anti-cardiolipin / antiphospholipid antibodies.

ASSAY PRINCIPLE:

The diluted assayed plasma sample or biological fluid is introduced into one of the microwells of the Cardiolipin coated plate. When present, anti-Cardiolipin / anti-phospholipid antibodies bind to immobilised and saturated Cardiolipin. Following a washing step, bound antibodies, of the IgA isotype, are revealed with a goat anti-human IgA (α specific)-peroxidase conjugate, which reacts specifically with IgA isotypes. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H_2O_2), is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with sulfuric acid. The colour developed is directly proportional to the amount of anti-Cardiolipin / anti-phospholipid antibodies, of the IgA isotype, present in the tested sample.

REAGENTS:

COAT : **Micro ELISA plate,** containing 12 strips of 8 wells, coated with anionic phospholipids, saturated, then stabilized; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
SD : 2 vials containing 50 mL of **Autoimmunity Sample Diluent**, ready to use. Contains Sodium Azide
CAL : 3 vials of **Anti-Cardiolipin, IgA, calibrator**, lyophilised. When restored with **1 mL** of **Autoimmunity Sample Diluent**, the ready to use calibrator is obtained (already diluted 1:100).
C- : 3 vials of **negative control**, lyophilised (diluted normal human plasma). When restored with **1 mL** of **Autoimmunity Sample Diluent**, the ready to use negative control is obtained (already diluted 1:100).
IC : 3 vials of **immunoconjugate (Anti-IgA-HRP immunoconjugate)**, affinity purified goat antibodies specific for human IgA-α specific coupled to HRP, lyophilised.
CD : 1 vial of 25 mL of **conjugate diluent**, ready to use.
WS : 1 vial of 50 mL of **Wash Solution**, 20 fold concentrated.
TMB : 1 vial of 25 mL peroxidase substrate: 3,3',5,5' **– Tetramethylbenzidine** containing hydrogen peroxide, ready to use.
SA : 1 vial of 6 mL of **0.45M Sulfuric Acid (Stop Solution)**, ready to use.

The exact concentration (expressed in APL units according to the KAPS standards) of control is indicated on the flyer provided in the kit. The concentration may slightly vary from lot to lot. For the assay, refer to the values indicated on the flyer provided in the kit used.
Reagent SD contains low concentration of Sodium azide (0.9 g/L) and reagent SA contains sulfuric acid, see CAUTIONS AND WARNINGS

CAUTIONS AND WARNINGS:

- Any product of biological origin must then be handled carefully, as being potentially infectious.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
- The disposal of waste materials must be carried out according to current local regulations
- Use only reagents from kits with the same lot number. Do not mix reagents from kits with different lots when running the assay; they are optimized for each lot of kits.
- Reagents must be handled with care, in order to avoid any contamination during use. Take care to limit as much as possible any evaporation of the reagents during use, by limiting the liquid-air surface exchange.
- In order to preserve the stability of the reagents, close the vials with their original screw cap following each use.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- For in vitro diagnostic use.
- Sulfuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle Sulfuric acid with great care. Wear protection glasses and gloves when handling. Avoid any skin and eye contact.

PREPARATION AND STABILITY OF REAGENTS:

Bring the kit at room temperature, at least 30 min before use. Store the unused reagents at 2-8°C. Vials are closed under vacuum. Remove carefully the stopper, in order to avoid any loss of powder when opening the vials.
When appropriately used and stored, according to the recommended protocol and cautions, the kit can be used over a two month period, and strip by strip, if required.
COAT (Micro ELISA plate): Open the aluminium pouch and take off the required amounts of 8 well strips for the test series. When out of the pouch, the strips must be used within 30 minutes. Unused strips can be stored at **2-8°C for 4 weeks** in their original aluminium pouch, in presence of the desiccant, hermetically closed and protected from any moisture, and stored in the provided plastic microplate storage bag (minigrip).
SD (Autoimmunity Sample Diluent): Ready to use. This reagent contains sodium azide. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:
- **4 weeks** at 2-8°C

CAL (Calibrator): Reconstitute each vial with **1 mL** of **"Automimmunity Sample Diluent"**, shake thoroughly for complete dissolution. The obtained calibrator is ready to use and it corresponds to a plasma containing IgA isotype antibodies to cardiolipin, already **diluted 1:100**.
Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:
- **5 days** at 2-8°C.

C- (Negative Control): Reconstitute each vial with **1 mL** of **"Autoimmunity Sample Diluent"**, shake thoroughly for complete dissolution. The obtained negative control is ready to use and it corresponds to a normal human plasma, already **diluted 1:100**.
Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
- **2 weeks** at 2-8°C.

IC (Anti-IgA-HRP immunoconjugate): Reconstitute each vial with **7.5 mL of Conjugate Diluent** at least 15 min before use. Let the pellet to be completely dissolved before use, and shake the vial gently in order to homogenize the content.
Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
- **4 weeks** at 2-8°C.
- **24 hours** at room temperature (18-25°C).

CD (Conjugate Diluent): Ready to use. This reagent contains 0.05% Kathon CG. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
- **4 weeks** at 2-8°C

WS (Wash Solution): Incubate, if necessary, the vial in a water bath, at **37°C**, until complete dissolution of crystals. Shake the vial and dilute the amount required **1:20** in distilled water (the 50 mL contained in the vial allow to prepare 1 liter of Wash Solution).
Stability of the wash solution, provided that any contamination or evaporation is avoided, kept in its original vial:
- **8 weeks** at 2-8°C

Stability of the dilute wash solution, provided that any contamination or evaporation is avoided, kept in its original vial:
- **When open, 7 days** at 2-8°C

This reagent contains 0.05% Kathon CG.
TMB : Ready to use. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
- **4 weeks** at 2-8°C

SA (Stop Solution): Stop solution containing 0.45M sulfuric acid, ready to use.

STORAGE CONDITIONS:

Unopened reagents must be stored at 2-8°C, in their original packaging box. They are then usable until the expiration date printed on the label.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:
- Distilled water.

Materials:
- **8-channel** or **repeating pipette** allowing dispensing volumes of 50-300 µL**.**
- **1-channel pipettes** at variable volumes from 0 to 20 µL, 20 to 200 µL and 200 to 1000 µL.
- **Micro ELISA plate washing equipment** and **shaker**.
- **Micro ELISA plate reader** with a wavelength set up at 450 nm.

SPECIMEN COLLECTION:

Preparation and storage of specimens must be performed according to the current local regulations.
- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate). EDTA collected human plasma may also be used.
- Collection:

Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M), with caution, through a net venipuncture. The first tube must be discarded.
- Centrifugation:

Within 2 hours, use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2500 g at room temperature (18-25°C) and plasma must be decanted into a plastic tube.
- Storage of plasma:
  - 4 hours at room temperature (18-25°C)
  - 48 hours at 2-8°C.
  - 1 month at -20°C.

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

TEST PROCEDURE:

Assay procedure:

1. Calibrator and Negative Control are ready to use (**already diluted 1:100**)

2. The samples should be diluted using Automimmunity Sample Diluent (SD) as described in the table below:

Samples	Dilution
Plasma	1:100
Serum	1:100
Biological fluid	1:100

When high amounts of anti-cardiolipin / anti-phospholipid antibodies are expected, dilute at **1:200** or **1:400** dilutions. Results must then be multiplied by **2** or **4**.

3. Remove the required number of strips from the aluminium pouch and put the strips in the frame provided. In the different wells of the micro ELISA plate, introduce the reagents and perform the various assay steps as indicated on the following table:

Reagent	Volume	Procedure
Anti-Cardiolipin IgA Calibrator dilutions or **Negative control** or **1:100 diluted sample** or **sample diluent** (blank)	**200 µL**	Introduce the : - **Calibrator dilutions** or - **Negative control** or - **Diluted sample** or - **Sample diluent** into the micro ELISA plate wells (a)
Incubate for 30 minutes at room temperature (18-25 °C) (b) (c)		
Wash Solution (20 fold diluted in distilled water)	**300 µL**	Proceed to 5 successive washings using the washing instrument **(c).**
Conjugate (anti-IgA-HRP immunoconjugate, restored with 7.5 mL of conjugate diluent)	**200 µL**	Immediately after the washing, introduce the anti-IgA-HRP immunoconjugate in the micro ELISA plate wells.
Incubate for 30 minutes at room temperature (18-25 °C) (b)		
Wash Solution (20 fold diluted in distilled water)	**300 µL**	Proceed to 5 successive washings using the washing instrument **(c)**.
TMB/H_2O_2 Substrate	**200 µL**	Immediately after the washing, introduce the substrate into the wells. **Nota**: The substrate distribution, row by row, must be accurate and at exact time intervals **(d)**
Let the colour develop for exactly **5 min**. at room temperature (18-25 °C) **(b)**		
0.45M Sulfuric Acid	**50 µL**	Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing the **0.45M** Sulfuric Acid **(d)**
Wait for **10 minutes** in order to allow the colour to stabilize and measure absorbance at **450 nm (A450)**. Substract the blank value. **(e)**		

Remarks:

a) Distribute calibrators, controls and tested specimen as rapidly as possible (within 10 minutes), in order to obtain an homogeneous immunological kinetics for antibodies binding. A too long delay between the distribution of the first and the last wells may induce an influence of immunological kinetics and produce wrong results.

b) Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used.

c) Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized components. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted in order to wash the plates gently, and to avoid a too drastic emptying, which could lower plate reactivity.

d) For addition of the TMB substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction.

e) For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

VALIDATION:

- Calibrator and controls provided in the kit allow validating the right performance of the assay.
- Expected A450 values for undiluted calibrator and negative controls can present variations from lot to lot but, when the assay is performed at room temperature, between 18 and 25°C, they always are:

P = A450 for 1:1 calibrator: ≥ 1.5 **N = A450 for negative control: ≤ 0.25**

In addition, concentrations obtained for controls must be within the acceptance ranges indicated on the flyer provided in the kit. If controls are out of these ranges check carefully the assay conditions and re-run the assay, if required.

CALIBRATION:

The assay can be calibrated with the calibrator provided in the kit, and which concentration (C) is indicated in APL units (APL), on the flyer provided. Prepare the standard solutions for calibration by doing serial two-step dilutions of the calibrator in Autoimmunity Sample Diluent, from 1:1 to 1:32. A concentration range from C:1 to C:32 is obtained.

- The usual dynamic range is from 0 to about 80 APL units.

RESULTS:

- Results are expressed according to the A450 values obtained for samples, and controls and anti-cardiolipin / anti-phospholipid concentrations are calculated using the calibration curve.
- The calibration curve is obtained by plotting the anti-cardiolipin concentrations of calibration range expressed in **APL** on the abscissae and the corresponding **A450** on the ordinates. The anti-Cardiolipin, antibody concentration, of the IgA isotype, obtained for the sample tested at the standard **1:100** dilution, and expressed in APL units, is directly deduced from the curve.
- When higher dilutions are used, (i.e. **D**), the concentration measured must be multiplied by the complementary dilution factor (i.e. **D:100** ; for example **x2** for **1:200** or **x4** for **1:400**), etc…
- Alternatively, an ELISA software (i.e. DYNEX, BIOLISE, etc…), can be used for the calculation of anti-cardiolipin/anti-phospholipid antibody concentrations.

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

LIMITATIONS:

- In order to get the optimal performances of the assay, the technical instructions must be strictly followed.
- Any reagent presenting an unusual aspect or contamination signs must be rejected.
- Any plasma containing a coagulum or contamination signs must be rejected.
- If the washing step is not correctly performed, the negative control can produce a high absorbance value. In order to avoid non-specific colour development, check that the washing step is performed efficiently.

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SYMBOLS:

Used symbols and signs listed in the ISO standard 15223-1.