ZYMUTEST ACA-APA
IgG - Isotype
Ref RK029A (96 tests)

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

Not for Sale in the US

INTENDED USE:
The ZYMUTEST ACA-APA, IgG ELISA kit, is an optimized enzyme immuno-assay designed for measuring anti-cardiolipin / anti-phospholipid antibodies of the IgG isotype, in human plasma or serum, or in any biological fluid where these antibodies must be measured.

SUMMARY AND EXPLANATION:
The ZYMUTEST ACA-APA, IgG Kit, specifically measures human anti-cardiolipin / anti-phospholipid antibodies of the IgG isotype, reactive with immobilised and saturated Cardiolipin. IgG or IgG isotypes are measured. These isotypes can be assayed with the ZYMUTEST ACA-APA IgM or ZYMUTEST ACA-APA IgA.

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 / anti-phospholipid antibodies.

ASSAY PRINCIPLE:
The diluted assayd 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 IgG isotype, are revealed with a goat anti-human IgG (Fc-specific) peroxidase conjugate, which reacts specifically with IgG isotypes. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H₂O₂), is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with sulfuric acid.

PREPARATION AND STABILITY OF REAGENTS:

Be 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 for a minimum period of 2 years. Do not use a reagent if it has passed beyond its expiration date.

COAT (Micro ELISA plate): Open the aluminium pouch and take off the required amounts of 7.5 mL of Conjugate Diluent. 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 (mengrip).

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:
- 5 days at 2-8°C.
- 2 weeks at 2-8°C.

CAL (Calibrator): 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 is:
- 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 is:
- 4 weeks at 2-8°C.
- 8 weeks at 2-8°C.

Stability of the wash solution, provided that any contamination or evaporation is avoided, kept in its original vial is:
- 5 weeks at 2-8°C.

Stability of the dilute wash solution, provided that any contamination or evaporation is avoided, kept in its original vial is:
- 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 is:
- 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
- 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 (in the USA, refer to CLSI Document GP44-A4 for further instructions on specimen collection, handling and storage).

- **Specimens:**
  - Human plasma obtained from anticoagulated blood (trisodium citrate). EDTA collected human plasma may be used.
  - Collection:
    - Blood (9 vol.): Must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M), with caution, through a needle 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:
    - o 4 hours at room temperature (18-25°C).
    - o 48 hours at 2-8°C.
    - o 1 month at -20°C.

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

Interpretation of results:

A single and standardized calibrator is used for the assay calibration and the calibration range is obtained using a serial two-fold dilution. This ensures a higher reliability of the assay, and a higher accuracy and reproducibility from lot to lot, and run to run, for the cut-off.

Negative range: The calibrator expressed in GPL units is directly deduced from the calibration range expressed in GPL on the x-axis and the corresponding A450 on the y-axis. The anti-Cardiolipin, antibody concentration, of the IgG isotype, obtained for the sample tested at the standard 1:100 dilution, and expressed in GPL units, is directly deduced from the curve.

When higher dilutions are used (i.e. D), the concentration measured must be multiplied by the common dilution factor (i.e. D100; for example x2 for 1:200 or x4 for 1:400).

Alternatively, an ELISA software (i.e. DYNEX, BIOLiSE, etc...), can be used for the calculation of anti-cardiolipin/anti-phospholipid antibody concentrations.

Pathological Variations

Anti-cardiolipin/anti-phospholipid antibodies are usually absent in normal population. Presence of anti-cardiolipin/anti-phospholipid antibodies at moderate or high concentrations is observed in the antiphospholipid syndrome (APS), sometimes associated with thrombotic diseases, recurrent miscarriages, livedo reticularis, thrombocytopenia or neurological disorders.

The pathogenicity of anti-cardiolipin/anti-phospholipid antibodies is still under investigation. They are thought to contribute triggering various clinical manifestations (APS). Pathogenicity of the various isoforms is not completely documented, especially for IgM and IgG isotypes. Severity of the clinical manifestations associated with the presence of anti-cardiolipin/anti-phospholipid antibodies, increases with the IgG isotype, the antibody concentration and its affinity, and the time of exposure. IgG isotype is then, the most pathogenic.

Applications

Assay of anti-cardiolipin/anti-phospholipid antibodies of the IgG isotype, in the following clinical situations:

- Anti-phospholipid syndrome (APS).
- Pregnancies with recurrent miscarriage.
- Unexplained thrombosis.
- Any clinical situation where the assay of anti-Cardiolipin/anti-phospholipid antibodies is required.

Performances

- The lower limit of detection is ≤ 2 GPL/mL
- Inter assay: <10% 
- Intra assay: <10% 
- 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 the calibration range expressed in GPL on the x-axis and the corresponding A450 on the y-axis. The anti-Cardiolipin, antibody concentration, of the IgG isotype, obtained for the sample tested at the standard 1:100 dilution, and expressed in GPL units, is directly deduced from the curve.
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