Anti Cardiolipin IgM

NB-06-0579

FOR RESEARCH USE ONLY

Enzyme immunoassay for the quantitative determination of IgM auto-antibodies against Cardiolipin in human serum or plasma

1. INTENDED USE

Anti Cardiolipin IgM is an indirect solid phase immunoassay kit for the quantitative measurement of IgM class auto-antibodies directed against Cardiolipin-β2-glycoprotein complex in human serum or plasma. The assay is intended for research use only. Anti Cardiolipin IgM kit is intended for research use only.

2. PRINCIPLE OF THE ASSAY

Anti-Cardiolipin IgM test is based on the binding of antibodies on human serum or plasma directed against the antigenic complex between Cardiolipin and β2-Glycoprotein; this complex is coated on the microplate. The antibodies present in calibrators, controls or prediluted samples bind to this antigen. After a 60 minute incubation the microplate is washed with wash buffer to removing non-reactive serum or plasma components. An anti-human IgM horseradish peroxidase conjugate solution recognizes IgM class antibodies bound to the immobilized antigens. After a 60 minute incubation any excess enzyme conjugate, which is not specifically bound, is washed away with wash buffer. A chromogenic substrate solution containing TMB is dispensed into the wells. After a 15 minute incubation the color development is stopped by adding the stop solution. The solution turns yellow at this point. The level of color is directly proportional to the concentration of IgM antibodies present in the original sample.

3. MATERIALS

3.1. Reagents supplied

Anti-Phospholipid Coated Wells: 12 breakapart 8-well snap-off strips coated with antigenic Cardiolipin/ -2-Glycoprotein complex; in resealable aluminum foil.

Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact), ready to use

Conjugate: 1 bottle containing 15ml Anti h-IgM conjugate with horseradish peroxidise (HRP), BSA 0,1%, Proclin < 0,0015%

TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine 0,26 g/L, hydrogen peroxide 0,05%

Wash solution: 1 bottle containing 50 ml (10x conc.) Phosphate buffer 0,2M, proclin < 0,0015%

Sample Diluent: 1 bottle containing 100 ml Phosphate buffer 0,1M, NaN_3 < 0,1%

Anti-Cardiolipin IgM Standards: 5 bottles, 1, 2
ml each, ready to use Standard 0: 0 AU/ml
Standard 1: 5 AU /ml
Standard 2: 10 AU /ml
Standard 3: 20 AU /ml
Standard 4: 80 AU /ml
Positive Control: 1 bottle containing 1,2 ml, Phosphate buffer 0,1M,NaN3 < 0,1%, human serum, ready to use
Negative Control: 1 bottle containing 1,2 ml, Phosphate buffer 0,1M,NaN3 < 0,1%, human serum, ready to use

3.2. Materials supplied

1 Strip holder
1 Cover foils
1 Test protocol
1 Distribution and identification plan

3.3. Materials and Equipment needed

ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm Manual or automatic equipment for rinsing wells
Pipettes to deliver volumes between 10 and 1000 µl Vortex tube mixer

Distilled water
Disposable Tubes
Timer

4. STABILITY AND STORAGE

Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed Unused antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.

5. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!

5.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with antigenic Cardiolipin/B-2-Glycoprotein complex. Store at 2…8 °C. Open the bag only when it is at room temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminum foil along with the desiccant supplied and stored at 2…8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.*

5.2. Anti-Cardiolipin IgM Standards / control

The standards are ready to use and have approximately the following concentrations:

	S0	S1	S2	S3	S4
AU/mL	0	5	10	20	80

If stored at 2-8°C the standards are stable until expiration date.

Positive Control: 1 bottle containing 1,2 ml,Phosphate buffer 0,1M,NaN3 <

0,1%, human serum,ready to use Negative Control: 1 bottle containing 1,2

ml,Phosphate buffer 0,1M,NaN3 < 0,1%, human serum,ready to use

5.3. TMB Substrate Solution

The bottle contains 15 ml of 3,3',5,5'-tetramethylbenzidine 0,26 g/L, hydrogen peroxide 0,05%. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away*.

5.4. Stop Solution

The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

5.5. Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (10x) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C. In concentrated wash solution it is possible to observe the presence of crystals. In this case mix at room temperature until complete dissolution of crystals is observed. For greater accuracy dilute the whole content of the bottle of concentrated wash solution to 500 mL, taking care also to transfer crystals completely by rinsing of the bottle, then mix until crystals are completely dissolved.

5.6. Sample Diluent

The bottle contains 100 ml Phosphate buffer 0,1M, NaN3 < 0,1%

5.7. Conjugate IgM

The bottle containing 15ml Anti h-IgM conjugate with horseradish peroxidise (HRP), BSA 0,1%, Proclin < 0,0015%

6. SPECIMEN COLLECTION AND PREPARATION

Either human serum or plasma samples can be used for the test execution. Test samples should be clear. Contamination by lipemia is best avoided, but does not interfere with this assay. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months. Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of autoantibody activity. Testing of heat-inactivated sera is not recommended. All serum and plasma samples have to be diluted 1:100 with sample diluent. Therefore 10 L of sample may be diluted to 1,000 L with sample diluent. The Controls are ready to use.

7. ASSAY PROCEDURE

7.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

1 well (e.g. A1) for the substrate blank 2 wells
(e.g. B1+C1) for standard 0 2 wells (e.g. D1+E1)
for standard 1 2 wells (e.g. F1+G1)
for standard 2 2 wells (e.g. H1+A2) for
standard 3 2 wells (e.g. B2+C2) for standard 4
2 wells (e.g. D2+E2) for positive control 2
wells (e.g. F2+G2) for negative control

It is *recommended to determine standards and samples in duplicate.*Perform all assay steps in the order given and without any appreciable delays between the steps.A clean, disposable tip should be used for dispensing each standard and each sample.

7.2. Test Procedure

Reagents	Standard	Sample or Controls	Blank
Standard S0-S4	100 µL		
Controls		100 µL	
Diluted Sample		100 µL	
Incubate for 60 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells three times with 300 µL of diluted wash solution .			
Conjugate	100 µL	100 µL	
Incubate for 60 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells three times with 300 µL of diluted wash solution.			
TMB substrate	100 µL	100 µL	100 µL
Incubate for 15 minutes in the dark at room temperature (22-28°C).			
Stop solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank			

8. QUALITY CONTROL

The anti-Cardiolipin Positive and Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
Because the Positive and Negative Controls are prediluted, do not use procedural methods associated with dilution of specimens. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at < -20°C.
For best results, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated: -The Positive and Negative Controls are intended to monitor for substantial reagent failure and they will not ensure precision at the assay cut-off. -This test is only valid if the optical density at 450 nm for the Positive Control as well as for the Calibrator (S0-S4) complies with the respective range indicated on the Quality Control Certificate enclosed in each test kit.

9. RESULTS

For Anti-Cardiolipin a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable. However a Lin-Log Plot is recommended. First calculate the average optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

10. LIMITATIONS OF PROCEDURE

The presence of immune complexes or other immunoglobulin aggregates in the sample may cause an increased level of non-specific binding and produce false positives in this assay.

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Precision

Precision and reproducibility are evaluated by two positive samples tested in two different runs with two different lots. Dispensing and washing operations were performed manually by an operator.The results in terms of standard deviation and coefficient of variation were below:

	IgM			
Sample	1		2	
	SD	CV%	SD	CV%
Intra-assay	3,36	10,5	2,21	10,1
Inter-assay	0,20	9,3	4,17	10,3

11.2. Sensitivity

Test against a commercial reference kit, performed on 41 sera (including 18 positive sera and 23 negative sera) showed a sensitivity of 81.8%.

11.3. Specificity

Test against a commercial reference kit, performed on 41 sera (including 18 positive sera and 23 negative sera) showed a specificity of 100.0%.

11.4. Detection limit

The lowest concentration of anti-Cardiolipin IgM that can be distinguished from zero standard is 0.12 AU/mL with a confidence limit of 95%.

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

13. WARNINGS AND PRECAUTIONS

This kit is intended for research use only by professional persons. Use appropriate personal protective equipment while working with the reagents provided. All human source material used in the preparation of standards and controls for this product has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Standard and the Controls should be handled in the same manner as potentially infectious material. Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious. Some reagents contain small amounts of Sodium Azide (NaN3) or Proclin 300R as preservatives. Avoid the contact with skin ormucosa. Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing toform potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up. The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes. The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes. Avoid the exposure of reagent TMB/H2O2 to directed sunlight, metals or oxidants.

PRECAUTIONS

Please adhere strictly to the sequence of pipetting steps provided in this protocol. All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use. Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must be observed. Do not use any kit component beyond their expiry date. WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly; therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, for doses dispensed with the aid of automatic and semi-automatic devices, before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips. If you use automated equipment is your responsibility to make sure that the kit has been appropriately tested. The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the back ground. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction. Observe the guidelines for performing quality control in laboratories by assaying controls and/or pooled sera. Maximum precision is required for reconstitution and dispensation of the reagents. Samples microbiologically contaminated should not be used in the assay. Highly lipemeic or haemolysed specimens should similarly not be used Plate readers measure vertically. Do not touch the bottom of the wells.

14.BIBLIOGRAPHY

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SCHEME OF THE ASSAY

Anti-Cardiolipin IgM

Test Preparation

Prepare reagents and samples as described.Establish the distribution and identification plan for all specimens and controls on the resultsheet supplied in the kit.

Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

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