Version 22-04/24

User's Manual

Human Cardiolipin IgM ELISA Kit

REF **DEIA1708M**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Enzyme immunoassay for the qualitative and quantitative determination of IgM antibodies against cardiolipin.

General Description

Cardiolipin IgM ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for the quantitative or qualitative determination of IgM class antibodies directed against Cardiolipin in human serum or plasma samples. Its function is the aid to diagnosis of anti-phospholipid syndrome (APS) and APS associated with systemic lupus erythematosus (SLE).

Principles of Testing

The wells of the solid phase are coated with CL and ß2-GP1. On this surface, the following immunological reactions take place:

1. CL/ß2-GP1-specific antibodies present in the sample bind to the immobilized antigen, forming the antigen-antibody complex. Then, non-bound sample components are washed away from the solid phase.
2. A second antibody, directed at human IgM antibodies and conjugated with horse-radish peroxidase (HRP), is added. This conjugate binds to the complex. Then, excess conjugate is washed away from the solid phase.
3. The enzyme-labelled complex converts a colorless substrate into a blue product. The degree of color development reflects the concentration of CL/ß2-GP1 IgM in the sample.

Reagents And Materials Provided

1. **Microtiter Plate:** coated with CL/ß2-GP1 and hermetically packed in a foil laminate pouch together with a desiccant bag. The plate consists of 12 strips, each of which can be broken into 8 individual wells.
2. **Enzyme Conjugate IgM:** 14 mL, ready-to-use, green colored. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane.
3. **Calibrator A-F:** 2.0 mL each, 0 - 3.0 - 8.0 - 18 - 45 and 100 MPL-U CL/ß2-GP1 IgM / mL, ready-to-use, gradually blue colored. Contain TBS, BSA, Tween and Na-azide.
4. **Negative and Positive Control:** 2.0 mL each, ready-to-use, green and red colored, respectively. Contain TBS, BSA, Tween and Na-azide.
5. **Sample Diluent:** 100 mL, ready-to-use, orange colored. Contains Tris-buffered saline (TBS), bovine serum albumin (BSA), Tween and Na-azide.
6. **TMB Substrate Solution:** 14 mL, ready-to-use, colorless. Contains a buffered solution of TMB and H_2O_2. Contained in a vial impermeable to light.
7. **Wash Buffer:** 100 mL, 10× concentrate, blue colored. Contains TBS, Tween and bromonitrodioxane.
8. **TMB Stop Solution (0.2 M H_2SO_4):** 14 mL, colorless, ready-to-use. Caution: sulfuric acid is corrosive.
9. Instructions for Use.

10. Lot-specific certificate of analysis.

Materials Required But Not Supplied

1. Deionised or distilled water
2. Graduated cylinder, 1000 mL
3. Tubes for sample dilution (transfer tubes in the microwell plate format recommended)
4. Pipettes for 10, 100 and 1000 µL (1- and 8-channel pipettes recommended)
5. Microwell plate washer (optional)
6. Microwell plate photometer fitted with a 450 nm filter
7. ELISA evaluation program (recommended)

Storage

Store kit at 2 - 8°C, do not freeze. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

Reagent Preparation

Do not exchange or pool corresponding components from different kits, due to possibly different shipping or storage conditions. If the kit is to be used for several tests, only the currently needed amount of reagents should be withdrawn. It is crucially important that no cross-contamination between the reagents occurs. Use only clean pipettes and do not pour back residues into the original flasks.

1. The solid phase must reach room temperature before opening the pouch. Remove the supernumerary microwells from the frame and immediately put them back into the pouch, together with the desiccant bag. Reseal the pouch hermetically and keep it refrigerated for future use.
2. Dilute the wash buffer 10x-concentrate (100 mL, blue) with 900 mL deionised water. Mix thoroughly. The diluted buffer is stable for several weeks if stored refrigerated (2 - 8°C).
3. Preparation of the samples: handle patient specimens as potentially infectious agents. Besides serum, EDTA-, citrate- or heparin-treated plasma is suitable sample material as well.
4. Specimen requirements: highly lipemic, haemolysed or microbially contaminated samples may cause erroneous results and should be avoided.
5. Prepare samples using normal laboratory techniques. Turbid samples must first be clarified (centrifuged). The clarified or clear samples are mixed and then diluted 1/100, e.g. 10 µL serum or plasma + 990 µL sample buffer. Also mix the dilution.
6. For rapid dispensing during the assay procedure, preparation of the calibrators, controls and samples in microwell transfer tubes is recommended. This allows the operation of an 8-channel pipette during the assay procedure.
7. If samples are not assayed immediately, they should be stored at 2 - 8°C and assayed within 3 days. Repeated freezing and thawing of samples should be avoided. Thawed samples must be mixed prior to diluting.

Assay Procedure

Before starting the assay, all components of the kit must have reached room temperature (23 ± 3°C). To achieve best results, i.e. the maximum ratio between specific and background signal, careful washing is essential (steps 1, 3 and 5). It is **crucially important to remove the wash solution completely**. For that purpose, tap the plate firmly on several layers of absorbent tissue. Automated washers must be verified according to results obtained by manual washing.

1. Immediately prior to use, wash the solid phase once: fill wells with 350 µL wash buffer each, let soak for about 10 seconds in the wells and remove.
2. Dispense the calibrators (2.0 mL each, ready-to-use, gradually blue), controls (2,0 mL each, ready-to-use, green and red) and the diluted samples rapidly into the microwells; 100 µL per well. Duplicate measurements are recommended. Incubate the plate for 30 minutes at room temperature (23 ± 3°C).
3. Wash the wells 4 times as in step 1.
4. Rapidly (preferably using an 8-channel pipette) dispense the conjugate (14 mL, ready-to-use, green); 100 µ L per well. Incubate the plate as in step 2.
5. Repeat wash step 3.
6. Rapidly (preferably using an 8-channel pipette) dispense the substrate solution (14 mL, ready-to-use, colorless, black vial); 100 µL per well. Incubate the plate as in step 2. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.
8. Rapidly (preferably using an 8-channel pipette) dispense the stop solution (14 mL, ready-to-use, colorless. Caution: corrosive!); 100 µL per well. Use the same sequence as for the substrate. The color changes from blue to yellow. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.
9. Immediately read the absorbance in the microwell plate photometer at 450 nm.

Refrigerate the remainder of the reagents (2 - 8°C) if they are to be used again.

Interpretation Of Results

Based on the measurement of a blood donor and a positive collective of sera (see below), we suggest for the assessment of patient sera:

	quantitative evaluation MPL-U IgM / mL sample	qualitative evaluation ratio
normal (negative) range	< 5,0	< 0,87
cut-off	6,0	1,00
equivocal range	5,0 - 7,2	0,87 - 1,16
positive range	> 7,2	> 1,16

These specifications are given as an indication only; in order to check their accuracy, each analysis should include parallel samples of normal sera.

A negative test result indicates that the patient does not have an elevated level of IgM antibodies to CL/ß2-GP1. If characteristic clinical signs of APS are nevertheless observed, IgG/IgA antibodies directed at CL and/or antibodies directed at ß2-GP1 should be determined.

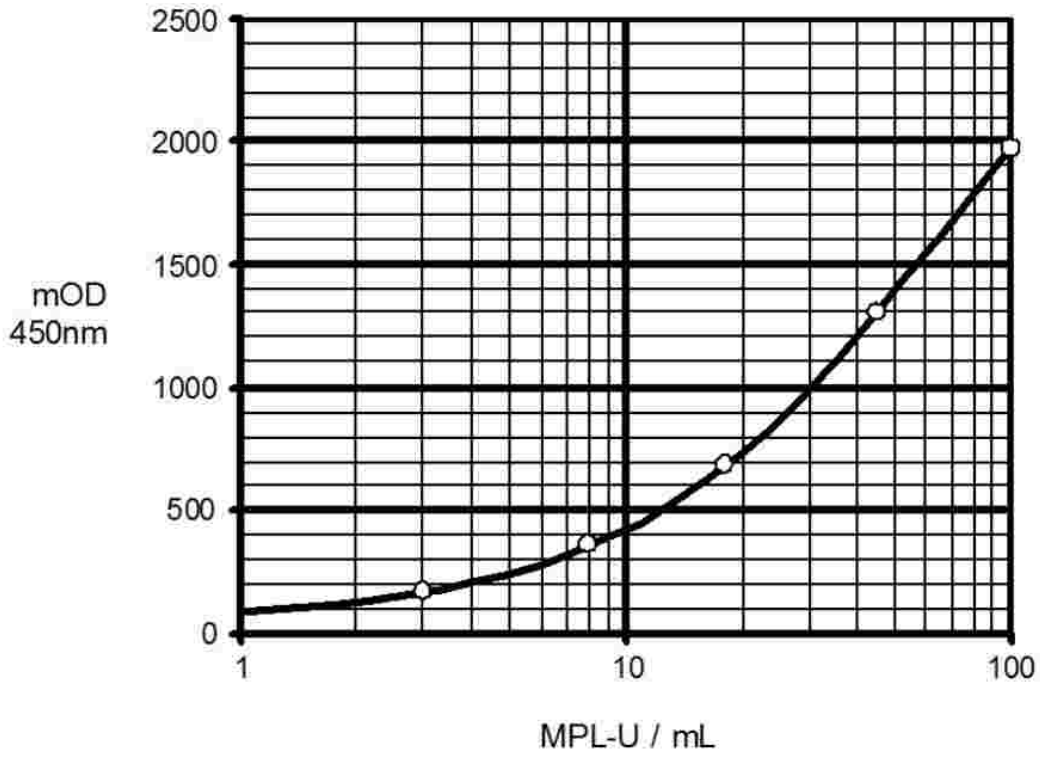
A positive result should be considered as an indication for APS, as outlined in the beginning. However, it

needs to be positive on at least two occasions, separated by 12 weeks, to be considered diagnostic for APS (1).

Specimens exhibiting results within the borderline range quoted above should be considered as equivocal and reported as such. It is recommended that a second sample be collected two weeks later and run in parallel with the first sample to document a possible change of antibody titer.

Evaluation

Quantitative evaluation: the data obtained are quantitatively evaluated with the standard curve, as shown below. However, the depicted curve can only serve as a model. It can not substitute the measurement of the calibrators, together with the controls and actual samples. The curve has been constructed with a conventional ELISA evaluation program, using a 4-parameter function. The Spline approximation is also appropriate.



If no computer-supported evaluation is possible, the standard curve may be drawn by hand. It allows transformation of the absorbance value of a sample into its concentration, i.e. into MPL-U CL/ß2-GP1 IgM per mL sample.

Qualitative evaluation: the test may also be evaluated in a qualitative manner. This requires measurement of the positive control only. Nevertheless, measurement and examination of the negative control is recommended (see below: quality control).

In qualitative test evaluation, the absorbance of the samples is compared with the borderline absorbance (= cut-off). It is determined according to the following formula:

$$OD_{borderline} = OD_{positive\ control} \times factor$$

The factor depends on the kit lot and is quoted in the lot-specific certificate of analysis which is included with

each test kit. Example:

ODpositive control = 1250 mOD

factor = 0.35

ODborderline = 1250 mOD × 0.35 = 438 mOD

In order to gain an impression of how positive a particular sample is for CL/ß2-GP1 IgM, one may calculate the ratio, according to the formula:

ratio = OD sample / OD borderline

Example:

ODborderline = 438 mOD

ODsample = 1480 mOD

ratio = 1480 mOD / 438 mOD = 3.4

Quality control: the positive and negative control check the assay performance. Their authorised values and acceptable ranges, respectively, are quoted in the lot-specific certificate of analysis. Values of the controls must fall within the indicated ranges; otherwise, the results of the assay are invalidated.

Performance Characteristics

1. **Standardisation**

The test is standardised with a purified serum preparation containing IgM antibodies specifically directed at CL/ß2-GP1. This preparation is calibrated against a set of commercially available, gradually positive sera ("Harris sera"; Louisville APL Diagnostics Inc., Louisville, KY, USA). The degree of sample reactivity is measured in MPL units (MPL-U CL/ß2-GP1 IgM / mL). 1 MPL-U/mL corresponds to the antigen binding capacity of a 1 µg/mL solution of IgM antibody affinity-purified from standard serum.

2. **Analytical specificity**

The test allows the specific determination of human IgM antibodies directed against CL/ß2-GP1. Interference with anticoagulants (EDTA, Citrat, Heparin) in samples has been tested and no interference effects have been observed.

3. **Detection limit (analytical sensitivity)**

The detection limit is defined as that concentration of analyte that corresponds to the mean absorbance of Sample Diluent plus 3-fold standard deviation (s). It was determined as < 0,5 MPL-U CL/ß2-GP1 IgM per mL sample (n = 24).

Recommended measuring range: 2 - 100 MPL-U CL/ß2-GP1 IgM per mL sample.

Specificity: 98%

Sensitivity: 100%

Precision

For the assessment of the test precision, the variability of results under the following conditions was determined:

a. within 1 assay and between 3 assays,

b. between 3 operators and

c. between 2 kit lots

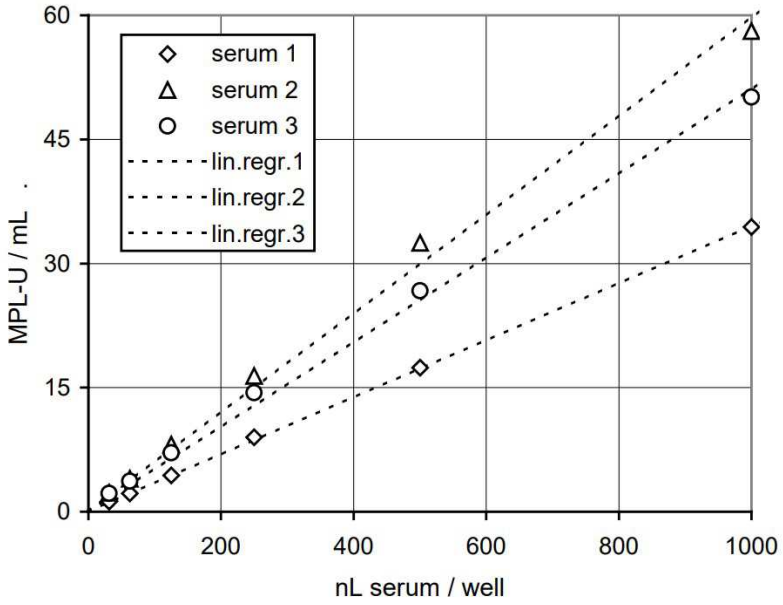
a. Intra- and inter-assay variability (n = 24 and 72, respectively)

b. Operator to operator variability (n = 12)

c. Variability between 2 kit lots (n = 6)

Linearity

In order to assess the dose-response relationship of the test, positive sera were measured in serial 2-fold dilution. Acceptance criterion: linear regression of 4 successive dilutions must yield a correlation factor > 0.98. A typical result is depicted below.



Precautions

1. It must be executed by trained professional staff.
2. The kit has been tested for transport stability and can be shipped unrefrigerated for up to 3 days. Store at 2 - 8°C on arrival. In case of doubt, contact your local distributor or the manufacturer.
3. Do not use reagents beyond their expiration dates.
4. Adherence to the protocol is strongly recommended.
5. The Sample Diluent, calibrators and controls contain Na-azide as antimicrobial agent. The wash buffer contains bromonitrodioxane and the conjugate methylisothiazolone / bromonitrodioxane as preservative. The substrate contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2). The stop solution, 0,2 M sulfuric acid (H_2SO_4), is acidic and corrosive.
6. The mentioned reagents may be toxic if ingested. Follow routine precautions for handling hazardous chemicals. Avoid all body contact, wear gloves and eye protection. If one of the reagents comes into contact

with skin or mucous membrane, wash thoroughly with water. Never pipette by mouth. Dispose in a manner complying with local/national regulations.

7. Na-Azide may react with lead and copper plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azide build-up.
8. The calibrators and controls contain components of human origin. They were tested for human immunodeficiency virus (HIV)-Ag, hepatitis B surface (HBs)-Ag and antibodies against HIV 1/2 and hepatitis C virus (HCV) and showed negative results; either in an FDA-approved or a CE-compliant test, according to European Directive 98/79/EC.
9. However, no test can guarantee that material of human origin is not actually infectious. The preparations should therefore be treated as potentially infectious and disposed of accordingly, as should the samples (and residues thereof); according to CDC (Center of Disease Control, Atlanta, USA) or other local / national guidelines on laboratory safety and decontamination.