



DCM111-3
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Anti beta 2 Glycoprotein 1 IgM

for routine analysis

Quantitative determination of IgM class antibodies against β 2-Glycoprotein I in human serum or plasma

IVD



LOT

See external label

2°C  8°C



$\Sigma = 96$ test

REF DKO111

INTENDED USE

[Anti Beta 2 Glycoprotein 1 IgM ELISA Assay Kit](#) is an indirect enzyme-linked immunosorbent assay (ELISA) designed for the quantitative measurement of IgM class antibodies directed against the β 2-Glycoprotein 1 in human serum or plasma. Anti beta 2 Glycoprotein 1 IgM ELISA Assay Kit is intended for research use only and is not intended for diagnostic procedures.

1. CLINICAL SIGNIFICANCE

The antiphospholipid syndrome (APS) is a disorder that presents peculiar symptoms: arterial and venous thrombosis, thrombocytopenia, ulcers of the lower limbs, hemolytic anemia, loss of the fetus during pregnancy and is associated with the presence of antiphospholipid antibodies. Antiphospholipid antibodies represent a large and heterogeneous immunoglobulins group, including anticardiolipin antibodies and lupus anticoagulant. The former are diagnosed by on their reactivity with cardiolipin or other anionic phospholipids in ELISA test, while the latter is detected in phospholipid-dependent coagulation tests (aPTT, KCT, dRVVT). In early '90s it was observed that antiphospholipid antibodies are not directed against anionic phospholipids, as long it was considered, but they react with plasma proteins bound to anionic (phospholipidic) surfaces. In fact about cardiolipin, it was observed the need of a cofactor for antibodies binding, this cofactor was identified in β 2 glycoprotein 1 (β 2-GP1). The β 2 glycoprotein 1 is a plasma glycoprotein of molecular weight of 50 kD, which is complexed to lipoproteins for 40%. A patient with clinical suspicion of APS, who has an high titre of anti-beta2GP1 antibodies, even if LAC or anti-cardiolipin antibodies are negative, has a strong chance of having the antiphospholipid syndrome, because these antibodies recognize β 2-GP1 bound to the surface of phospholipid (cardiolipin). These proteins seem to express their antigenicity only after contact with specific areas such as the anionic phospholipid surface or very hydrophilic plastic surfaces.

The importance of ELISA anti-phospholipids test, including the anti- β 2-GP1 test, lies in the fact that a positive test if associated with one or more of the symptoms can confirm a diagnosis of APS.

2. PRINCIPLE

Anti Beta 2 Glycoprotein 1 IgM ELISA Assay Kit is based on the initial binding of the antibodies present in calibrators, controls or pre-diluted samples to the beta 2 Glycoprotein 1 coated on the inner surface of the microplate wells. After a 60 minutes incubation the microplate is washed with a wash buffer to remove the non-reactive serum components.

Then an anti-human-IgM horseradish peroxidase conjugated solution recognizes IgM class antibodies bound to the immobilized antigens. After a 30 minutes incubation the excess of enzyme conjugate, which is not specifically bound, is washed away with the wash buffer.

Finally, a chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation 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. The concentration of IgM antibodies present in the sample is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

- Anti- β 2-GP1 Calibrators (5 vials, 1,2 mL each)
Phosphate buffer 0,1M, $\text{NaN}_3 < 0,1\%$, human serum
CAL0 **REF DCE002/11106-0**
CAL1 **REF DCE002/11107-0**
CAL2 **REF DCE002/11108-0**
CAL3 **REF DCE002/11109-0**
CAL4 **REF DCE002/11110-0**
- Controls (2 vials, 1,2 mL each, ready to use)
Phosphate buffer 0,1M, $\text{NaN}_3 < 0,1\%$, human serum
Negative Control **REF DCE045/11101-0**
Positive Control **REF DCE045/11102-0**
- Sample diluent (1 vial, 100 mL)
Phosphate buffer 0,1 M $\text{NaN}_3 < 0,1\%$ **REF DCE053-0**
- Conjugate (1 vial, 15 mL)
Anti human-IgM conjugate with horseradish peroxidase (HRP), BSA 0,1%, Proclin $< 0,0015\%$ **REF DCE002/11102-0**
- Coated Microplate
(1 breakable microplate coated with Beta-2-Glycoprotein 1) **REF DCE002/11103-0**
- TMB Substrate (1 vial, 15 mL)
3,3',5,5'-tetramethylbenzidine 0.26 g/L, hydrogen peroxide 0.05%, proclin $< 0,0015\%$ **REF DCE004-0**
- Stop Solution (1 vial, 15 mL)
Sulphuric acid 0,15M **REF DCE005-0**
- 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0,2 M, proclin $< 0,0015\%$ **REF DCE054-0**

3.2. Necessary Reagents not supplied

Distilled water

3.3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplate reader (450 nm).

4. WARNINGS

- This Anti Beta 2 Glycoprotein 1 IgM ELISA Assay Kit is intended for research use only by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the Anti Beta 2 Glycoprotein 1 IgM ELISA Assay Kit reagents 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, Calibrators and 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 of the Anti Beta 2 Glycoprotein 1 IgM ELISA Assay Kit contain small amounts of Sodium Azide (NaN₃) or Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form 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. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents of the Anti Beta 2 Glycoprotein 1 IgM ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange Anti Beta 2 Glycoprotein 1 IgM ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels 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.**

For this purpose, Diametra supplies a separate decontamination reagent for cleaning needles.

- If you use automated equipment, the user has the 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 background.
- 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 medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C₀...C₄)

Since no international reference preparation for anti beta 2 Glycoprotein 1 IgM antibodies is available, the assay system is calibrated in relative arbitrary units. The Calibrators are ready to use and have the following concentrations:

	C ₀	C ₁	C ₂	C ₃	C ₄
AU/mL	0	10	20	40	160

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of the Sample

For determination of anti beta 2 Glycoprotein 1 IgM antibodies human serum or plasma are the preferred sample matrixes.

All serum and plasma samples have to be pre-diluted with sample diluent 1:100; for example 10 µL of sample may be diluted with 990 µL of sample diluent.

The subjects need not to be fasting, and no special sample preparation is necessary. Collect blood by venipuncture into vacutainers and separate serum (after clot formation) or plasma from the cells by centrifugation.

Samples may be stored refrigerated at 2-8°C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20°C. To avoid repeated thawing and freezing the samples should be aliquoted.

Neither Bilirubin nor Hemolysis have significant effect on the procedure.

The controls are ready to use.

6.3. Preparation of the 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 bottle of concentrated wash solution to 500 mL taking care also to transfer the crystals completely, then mix until crystals are completely dissolved.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.**
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C₀-C₄), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Calibrator C ₀ -C ₄	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 3 times with 300 µL of diluted wash solution			
Conjugate	100 µL	100 µL	
Incubate for 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 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 within 5 minutes.			

8. RESULTS

8.1. Calibration curve

For Anti beta 2 Glycoprotein 1 IgM test 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 averaged 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.

Typical results (for example only)

The table below shows typical results for Anti- β2 glycoprotein 1 IgM. The data are for illustration only and should not be used to calculate the results.

N	OD1	OD2	mean	C1	C2	mean	CV%
CAL0	0.014	0.014	0.014	0.00	0.00	0.00	---
CAL1	0.297	0.302	0.300	9.91	10.09	10.00	1.22
CAL2	0.588	0.598	0.593	19.83	20.17	20.00	1.22
CAL3	1.101	1.141	1.121	39.16	40.85	40.01	3.00
CAL4	2.501	2.390	2.446	171.6	148.5	160.1	10.22

9. REFERENCE VALUES

In a normal range study with serum samples from healthy blood donors the following ranges have been established with Anti-Beta2 Glycoprotein 1 IgM test:

Anti beta 2 Glycoprotein 1 IgM (AU/mL)	
Negative	< 20
Positive	> 20

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works. It is recommended that each laboratory establishes its own normal and pathological ranges of seric Anti-β2-GP1 IgM.

9.1. Specificity

Test against two commercial reference kits, performed on 41 sera (including 15 positive and 26 negative) showed a specificity >99% (the first one) and of 95.8% (the second one)

9.2. Sensitivity

Test against two commercial reference kits, performed on 41 sera (including 15 positive sera and 26 negative sera) showed a sensitivity > 99% (the first one) and of 82.3% (the second one).

9.3. Detection limit

The lowest concentration of anti-beta 2 glycoprotein 1, which can be distinguished from zero Calibrator is 0.11 AU/mL with confidence limit of 95%.

9.4. Precision and reproducibility

9.4.1. Intra-Assay

Within run variation was determined by replicate 12 times three different sera with values in the range of calibration curve. The within assay variability is ≤ 6.1%.

9.4.2. Inter-Assay

Between run variation was determined by replicate the measurements of two different control sera with different lots of kits and/or different mix of lots of reagents. The between assay variability is $\leq 10.1\%$.

10. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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DiaMetra S.r.l. Headquarter: Via Garibaldi, 18 –
20090 SEGRATE (MI) Italy
Tel. 0039-02-2139184 – 02-26921595
Fax 0039-02-2133354.

Manufactory: Via Pozzuolo 14, 06038 SPELLO (PG) Italy
Tel. 0039-0742-24851
Fax 0039-0742-316197
E-mail: info@diametra.com

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com Info@eaglebio.com



ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed) too high within-run
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
- too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation