Anti beta 2 Glycoprotein 1 Screen `for routine analysis`

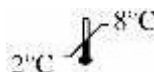**Quantitative determination of IgG o IgM class antibodies against β2-Glicoprotein 1 in human serum or plasma.**

IVD   ☣   LOT — See external label $2°C$ ⟋ $8°C$ $\Sigma = 96$ test   **REF** DKO145

INTENDED USE

Anti beta 2 Glycoprotein 1 Screen is an indirect enzyme-linked immunosorbent assay (ELISA) kit designed for the quantitative measurement of IgG or IgM class antibodies directed against the β2-Glycoprotein 1 in human serum or plasma.

Anti beta 2 Glycoprotein 1 Screen is intended for laboratory use only.

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 β2 glycoprotein 1 (β2-GP1). The β2 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 β2-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-β2-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

Dia.Metra Anti beta 2 Glycoprotein 1 Screen test allows to determine the unknown concentration of auto-antibodies directed against the β2-Glycoprotein 1 complex through two different calibration curve (one specific for IgG test, one specific for IgM test), two different conjugates linked to horseradish peroxidase (one specific for IgG test, the other specific for IgM test) and one microplate only. The principle of the method and the procedure are the same in both the tests. Use reagents for IgG or reagents for IgM depending on the isotype which is under investigation.

Anti beta 2 Glycoprotein 1 Screen test is based on the initial binding of antibodies present in calibrators, controls or pre-diluted patient 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 IgG (Conjugate IgG, reactive 3) or IgM (Conjugate IgM, reactive 6) horseradish peroxidase conjugated solution recognizes the IgG or IgM (respectively) 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 a wash buffer.

Finally a chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation colour development is stopped by adding the stop solution. The solution turns yellow at this point. The level of colour is directly proportional to the concentration of IgG or IgM antibodies present in the original sample.

The concentration of IgG or 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

- **Reagents for IgG class antibodies assay**

1. Anti β2 GP1 Calibrators IgG (5 vials, 1,2 mL each)
Phospate buffer 0,1M, NaN_3 < 0,1%, human serum
CAL0   **REF** DCE002/11006-0
CAL1   **REF** DCE002/11007-0
CAL2   **REF** DCE002/11008-0
CAL3   **REF** DCE002/11009-0
CAL4   **REF** DCE002/11010-0

2. Controls IgG (2 vials, 1,2 mL each, ready to use)
Phosphate buffer 0,1M, NaN_3 < 0,1%, human serum
Negative Control   **REF** DCE045/11001-0
Positive Control   **REF** DCE045/11002-0

3. Conjugate IgG (1 vial, 15 mL)
Anti human-IgG conjugate with peroxidase, BSA 0,1%,
Proclin < 0,0015%   **REF** DCE002/11002-0

- **Reagents for IgM class antibodies assay**

4. Anti β2 GP1 Calibrators IgM (5 flaconi, 1,2 mL ciascuno)
Tampone fosfato 0,1M, NaN_3 < 0,1%, siero umano
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

5. Controls IgM (2 flaconi, 1,2 mL ciascuno, pronti all'uso)
Tampone fosfato 0,1M, NaN_3 < 0,1%, siero umano
Controllo Negativo   **REF** DCE045/11101-0
Controllo Positivo   **REF** DCE045/11102-0

6. Conjugate IgM (1 flacone, 15 mL)
Anti human-IgM coniugato con perossidasi di rafano
(HRP), BSA 0,1%, Proclin < 0,0015%
   REF DCE002/11102-0

- **Common reagents**

7. Sample diluent (1 vial, 100 mL)
Phosphate buffer 0,1 M NaN_3 < 0,1%
   REF DCE053-0

8. Coated Microplate
(1 breakable microplate coated with Beta 2-Glycoprotein 1)
   REF DCE002/11003-0

9. TMB-Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
   REF DCE004-0

10. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0,15M *(avoid any skin contact)*
   REF DCE005-0

11. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0,2M pH 7.4   **REF** DCE054-0

3.2. Necessary Reagents not supplied
Distilled water

3.3. Auxiliary materials and instrumentation
Automatic dispenser.
Microplate reader (450 nm, 620-630 nm).

Note
Store all reagents at 2÷8°C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, the microplate is stable until the expiry date of the kit.

4. WARNINGS

- This kit is intended for in vitro use 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 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 contain small amounts of Sodium Azide (NaN_3) 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/H_2O_2 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 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 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, Dia.Metra 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. To improve the performance of the kit on automatic systems, it is recommended to increase the number of washes.
- 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 lipemeic 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 ($C_0...C_4$)
Since no international reference preparation for anti beta 2 Glycoprotein 1 Screen 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 Screen 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 patients 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 for 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.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- 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 (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Use reagents for IgG or reagents for IgM depending on the isotype which is under investigation			
Calibrator C_0-C_4 (IgG or IgM)	100 µL		
Controls (IgG or IgM)		100 µL	
Diluted Sample		100 µL	
Incubate for 60 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells 3 times with 300 µL of diluted wash solution.			
Important note: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.			
Automatic washer: if you use automated equipment, wash the wells at least 5 times.			
Conjugate (IgG or IgM)	100 µL	100 µL	
Incubate for 30 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells 3 times with 300 µL of diluted wash solution.			
Washing: follow the same indications of the previous point.			
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 a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

8. RESULTS

8.1. Calibration curve
For Anti beta 2 Glycoprotein 1 Screen kit 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 Screen. 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 beta 2 Glycoprotein 1 Screen test:

Anti beta 2 Glycoprotein 1 Screen (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 Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken on an individual patient basis.

It is recommended that each laboratory establishes its own normal and pathological ranges of seric anti beta 2 Glycoprotein 1 Screen.

10. PERFORMANCE AND CHARACTERISTICS FOR IgG TEST

10.1. Specificity
Test against two commercial reference kits, performed on 41 sera (including 14 positive and 27 negative) showed a specificity of 90% (the first one) and of 95.8% (the second one).

10.2. Sensitivity
Test against two commercial reference kits, performed on 41 sera (including 14 positive and 27 negative) showed a sensitivity >99% (the first one) and of 76.5% (the second one).

10.3. Detection limit
The lowest concentration of anti-beta 2 glycoprotein 1, which can be distinguished from zero Calibrator is about 0.47 AU/mL with confidence limit of 95%.

10.4. Precision and Reproducibility
10.4.1. 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 ≤ 7.0%
10.4.2. 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 ≤ 10.4%.

11. PERFORMANCE AND CHARACTERISTICS FOR IgM TEST

11.1. 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)

11.2. 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).

11.3. 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%.

11.4. Precision and reproducibility
11.4.1. 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%.

11.4.2. 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 ≤ 10.1%.

12. WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

1. Hughes GRV. The antiphospholipid Syndrome. Ten years on. Lancet 1993: 342; 341-344.
2. Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC, Brey R, Derksen R, Harris EN, Hughes GRV, Triplett DA, Khamashta MA. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. Arthritis Rheum 1999;42:1309-11.
3. Mc Neil HP, Simpson RJ, Chesterman CN, Krilis SA. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid binding inhibitor of coagulation: β2-Glycoprotein 1 (Apolipoprotein H). Proc Natl Acad Sci 1990;87:4120-4.
4. Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Koike T. Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. Lancet 1990;336:177-8
5. Pengo V, Biasiolo A, Fior MG. Autoimmune antiphospholipid antibodies are directed against a cryptic epitope expressed when β2-glycoprotein 1 is bound to a suitable surface. Thromb Haemost 1995:73:29-34
6. Arvieux J, Roussel B, Jacob MC, Colomb MG. Measurement of antiphospholipid antibodies by ELISA using β2-glycoprotein 1 as an antigen. J Immunol Methods 1991;143:223-9.
7. Tsutsumi A, Matsuura E, Ichikawa K, Fujisaku A, Mukai M, Kobayashi S, Koike T. Antibodies to β2-glycoprotein 1 and clinical manifestation in patients with systemic lupus erythemotosus. Arthritis Rheum 1996;39:1466-74.
8. Pengo V, Biasiolo A. The risk of overdiagnosis of antiphospholipid antibody syndrome. Throm Haemost 2001;86:933 (letter).

Ed. 09/2018   **DCM145-1**

Dia.Metra S.r.l.