



AccuDiag™ Beta 2 Glycoprotein 1 IgM ELISA

REF 1496-11

IVD See External Label 2°C - 8°C 96 Tests

Beta 2 Glycoprotein 1 IgM ELISA	
Principle	Indirect ELISA
Detection	Qualitative and Semi-Quantitative
Sample	5 µL serum/plasma
Incubation Time	~ 20 min.
Sensitivity	85.4%
Specificity	92.6%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The DAI Beta 2 Glycoprotein 1 IgM Enzyme-linked Immunosorbent Assay (ELISA) is intended for the detection and semi-quantitative determination of IgM antibodies to β_2 GP1 in human sera or plasma. The results of the assay are to be used as an aid in the diagnosis of certain autoimmune disease thrombotic disorders, anti-phospholipid syndrome, SLE or other lupus-like disorders.

SIGNIFICANCE AND SUMMARY

Cardiolipin autoantibodies (ACA) are described for various autoimmune diseases. The presence of anti-cardiolipin antibodies in systemic lupus erythematosus (SLE) can be related to the development of thrombocytopenia, in gynaecology they are supposed to cause intrauterine death or recurrent abortion. Furthermore, anti-cardiolipin antibodies have

been found in some non-thrombotic neurological disorders like cerebrovascular insufficiency, cerebral ischemia or chorea and in myocardial infarction. (1)

Recent studies have shown that a 50kD serum cofactor is required for anticardiolipin antibodies, to bind to cardiolipin which has been coated onto plastic plates. The cofactor has been identified as β_2 -glycoprotein 1 also termed apolipoprotein H. β_2 GP1 has been known as an in vitro inhibitor of the intrinsic blood coagulation pathway, ADP-dependent aggregation, and prothrombinase activity of activated platelets. (2-7)

It has become apparent that anticardiolipin antibody from patients with anti-phospholipid syndrome (APS) recognize a modified β_2 GP1 structure and not cardiolipin, native β_2 GP1 or an epitope structurally defined by both cardiolipin and β_2 GP1. (2-6)

Galli et al. (3) and Viard, et al. (8) reported that anti-cardiolipin antibody derived from SLE and APS were directed to the β_2 GP1 molecule coated on polystyrene plates. Koike and Matsuura showed conclusively that β_2 GP1 is indeed the antigen to which many anticardiolipin antibody patients are actually binding and furthermore showed that the phospholipid merely serves to link the β_2 GP1 to the solid phase. (2-9)

β_2 GP1 autoantibodies are found in the immunoglobulin classes IgG, IgM and IgA. The determination of IgM antibodies is a valuable indicator in the diagnosis of beginning autoimmune disease, whereas IgG and/or IgA antibodies will be found in progressive stages of manifested autoimmune disorders. IgA antibodies are often associated with IgG antibodies. The determination of IgA antibodies seems to have a greater validity in thrombosis and fetal loss. (10). Indications for determination of anti β_2 GP1 antibodies are: SLE, Thrombosis, Thrombocytopenia, Cerebral Ischemia, Chorea, Epilepsy, Recurrent Abortion and Intrauterine Death.

ASSAY PRINCIPLE

Purified β_2 GP1 antigens are coated on the surface of microwells. Diluted patient serum or plasma, and calibrators, are added to the wells. The Anti β_2 GP1 specific antibodies, if present, bind to the antigens. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM specific antibodies in the sample. The results are read by a microwell reader, and compared in a parallel manner with calibrators.

SPECIMEN COLLECTION & PREPARATION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits

- | | |
|----------------------------------------------------------------------------------------|----------------|
| 1. Microwell strips: β_2 GP1 antigen coated wells. | 12 x 8 wells |
| 2. Sample diluent: Yellow color solution | 50 ml / bottle |
| 3. Washing concentrate 50x. | 15 ml / bottle |
| 4. TMB Chromogenic Substrate: Amber bottle. | 12 ml / bottle |
| 5. Enzyme conjugate: | 12 ml / bottle |
| 6. Calibrator Stock. 100 SMU. | 160 µl / vial |
| 7. Control set: Negative and Positive controls.
Ranges are indicated on each label. | 160 µl / vial |
| 8. Stop solution. | 12 ml / bottle |



REAGENT PREPARATION

- Prepare 1x washing buffer.
Prepare washing buffer by adding distilled or deionized water to 50x wash concentrate to make a final volume of 750 ml.
- Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
- Preparation of Calibration Curve. It is recommended to use the calibrator set up within 24 hours.
For Calibrator A (100 SMU), add 10µl of calibrator stock to 1 ml of Sample Diluent. Prepare the Calibrators B, C, D, E and F by serial dilution of 500 µl of Calibrator A with equal volume of Sample Diluent.

Calibrator	Add	To Sample Diluent	SMU	
A	Calibrator Stock	10 µL	1000 µL	100
B	Calibrator A	500	500	50
C	Calibrator B	500	500	25
D	Calibrator C	500	500	12.5
E	Calibrator D	500	500	6.25
F	Calibrator E	500	500	3.13

SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample dilution 1:101 = 5 µl / 500 µl		
2	Washing buffer (3 times)	350 µl	
3	Diluted samples, controls & calibrators	100 µl	30 minutes
4	Washing buffer (3 times)	350 µl	
5	Enzyme conjugate	100 µl	30 minutes
6	Washing buffer (3 times)	350 µl	
7	TMB Chromogenic Substrate	100 µl	15 minutes
8	Stop solution	100 µl	
9	Reading OD 450 nm		

ASSAY PROCEDURE

- Place the desired number of coated strips into the holder.
PRE-WASH Coated Wells - Repeat washing three times with washing buffer.
- Prepare 1:101 dilution of test samples by adding 5 µl of the sample to 500 µl of Sample Diluent. Mix well.
- Dispense 100 µl of diluted sera, calibrator set & controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
- Remove liquid from all wells. Repeat washing three times with washing buffer.
- Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
- Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
- Dispense 100 µl of TMB Chromogenic Substrate into each well and incubate for 15 minutes at room temperature.
- Add 100 µl of Stop solution to stop reaction.
Make sure there are no air bubbles in each well before reading.
- Read O.D. at 450 nm with a microwell reader.

RESULTS

- Construct a standard curve by plotting O.D. 450 nm on the y-axis against the concentration of calibrator SMU values on the x-axis on a log-log graph paper or log-lin graph.
- Using the O.D. value of each specimen, determine the concentration from the standard curve.
- A typical example:

Calibrator Set	β2GP1 IgM (SMU)	O.D. 450 nm	O.D. 450 nm Mean	SD	CV %
Calibrator F	3.13	0.060 0.073	0.067	0.009	13.8
Calibrator E	6.25	0.135 0.135	0.135	0.000	0.00
Calibrator D	12.5	0.255 0.254	0.255	0.001	0.27
Calibrator C	25	0.494 0.496	0.495	0.001	0.28
Calibrator B	50	0.961 0.931	0.946	0.021	2.24
Calibrator A	100	1.700 1.606	1.653	0.066	4.02

QUALITY CONTROL

- The negative control and positive control should be run with every batch of samples tested and the concentration must be within the range stated on its label.
- The O.D. value of blank (Sample Diluent) must be lower than 0.150 and the O.D. value of calibrator 100 SMU must be greater than 0.750. Additional controls may be prepared from human serum specimens and kept under -20°C.

INTERPRETATION

Each laboratory is recommended to establish its own normal range based upon its own techniques, controls, equipments and patient population according to their own established procedures. The followings are a suggestive guideline.

Negative: < 15 SMU
 Low positive: 15 - 30 SMU
 Moderate positive: 30 - 70 SMU
 High positive: > 70 SMU

A positive result suggests the possibility of certain autoimmune disease thrombotic disorders. A negative result indicates no β2 GP1 IgM antibody or levels below the detection limit of the assay.

PERFORMANCE CHARACTERISTICS

Sensitivity, specificity, and accuracy:

A total of 75 samples were assayed with the DIAGNOSTIC AUTOMATION ELISA β2 GP1 IgM (X values) and with a reference ELISA (1) (Y values). The correlation equation is:

$$Y = 0.6327 X + 13.242 \quad R^2 = 0.9056 \quad (n = 75)$$

The results are summarized:

Reference ELISA		
N	P	Total



DIAGNOSTIC AUTOMATION ELISA β 2 GP1 IgM	N	25 (D)	7 (B)	32
	P	2 (C)	41 (A)	43
	Total	27	48	75

$$\text{Sensitivity} = A / (A+B) = 41 / (41 + 7) = 85.4\%$$

$$\text{Specificity} = D / (C+D) = 25 / (2 + 25) = 92.6\%$$

$$\text{Accuracy} = (A+D) / (A+B+C+D) = (41 + 25) / (41 + 7 + 2 + 25) = 66 / 75 = 88\%$$

Precision:

Statistic for CV, mean and SD were calculated for each of three samples from the results of 8 determinations in a single run for intra-assay. Inter assay precision was calculated from the result of 8 determinations of 8 different runs.

Intra-assay	n	Mean SMU	SD	% CV
Serum A	8	23.38	1.30	5.57
Serum B	8	40.25	1.98	4.92
Serum C	8	80.38	0.74	0.93

Inter-assay	n	Mean SMU	SD	% CV
Serum A	8	22.50	1.52	6.75
Serum B	8	41.32	2.13	5.15
Serum C	8	81.63	1.25	1.50

INTERFERENCE AND CROSS-REACTIVITY

1. High titer of anti β 2GP1 IgG and IgA does not decrease the testing sensitivity for the anti β 2GP1 IgM.
2. When a sample containing a high concentration of RF IgM in the presence of a high titer of anti β 2GP1 IgG, it may induce the potential of false positive.
3. DIAGNOSTIC AUTOMATION anti β 2GP1 IgM test does not cross-react with the following IgM positive samples tested: Toxo, Rubella, CMV, Chlamydia trachomatis, Dengue, Mumps, Measles, EBV VCA, H. pylori and RF.

LIMITATIONS OF THE ASSAY

1. Diagnosis cannot be made on the basis of anti β 2 GP1 results alone. These results must be used in conjunction with information from clinical evaluation and other diagnostic procedure.
2. When a high tier of anti β 2 GP1 IgM in the presence of a high tier of RF IgM in a certain sample, there is a potential to obtain a false IgM result.
3. The clinical significance of β 2 GP1 antibodies in diseases other than SLE is currently under investigation.
4. When negative anti β 2 GP1 titers are found in the presence of clinical indications, a lupus anticoagulant, anti-cardiolipin or other additional testing is indicated.
5. It is to be expected that some samples can be anti-cardiolipin positive yet anti β 2 GP1 negative. The anti β 2 GP1 test is a more specific marker of thrombotic risk. The anticardiolipin test can produce false positive results due to cross-reactivity with dsDNA or certain infectious disease antibodies.

PRECAUTIONS

1. Potential biohazardous materials:
The calibrator and controls contain human source components, which have been tested and found nonreactive for Hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B

virus, or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984

2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
5. To prevent injury and chemical burns, avoid contact with skin and eyes or inhalation and ingestion of the following reagents: Enzyme conjugate, TMB chromogenic substrate and Stop solution.

STORAGE CONDITIONS

1. Store the kit at 2 – 8°C.
2. Always keep microwells tightly sealed in pouch with desiccants. It is recommended to use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light during storage or usage.

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016



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Quality
Management for
Medical Devices
CERTIFIED

 Diagnostic Automation/Cortez Diagnostics, Inc.
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Date Adopted	2023-09
Brand Name	AccuDiag™
REF 1496-11	AccuDiag™- Beta 2 Glycoprotein 1 IgM ELISA
<input type="checkbox"/> EC <input type="checkbox"/> REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
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