AccuDiag™ Beta 2 Glycoprotein 1 IgG ELISA

REF 1495-11

IVD | See External Lab | 2°C – 8°C | 96 Tests

Beta 2 Glycoprotein 1 IgG ELISA	
Principle	Indirect ELISA
Detection	Quantitative
Sample	5 µL serum/plasma
Incubation Time	~ 65 min.
Sensitivity	91%
Specificity	92%
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 DIAGNOSTIC AUTOMATION ß$_2$GP1 IgG Enzyme-linked Immunosorbent Assay (ELISA) is intended for the detection and semiquantitative determination of IgG antibodies to ß$_2$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 lupus-like disorders.

SIGNIFICANCE AND SUMMARY

Anti- 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 ß$_2$-glycoprotein 1 also termed apolipoprotein H. ß$_2$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 ß$_2$GP1 structure and not cardiolipin, native ß$_2$GP1 or an epitope structurally defined by both cardiolipin and ß$_2$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 ß$_2$GP1 molecule coated on polystyrene plates. Koike and Matsuura showed conclusively that ß$_2$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 ß$_2$GP1 to the solid phase. (2~9)

Anti-ß$_2$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 ß$_2$**GP1** antibodies are: SLE, Thrombosis, Thrombocytopenia, Cerebral Ischemia, Chorea, Epilepsy, Recurrent Abortion and Intrauterine Death.

ASSAY PRINCIPLE

Purified ß$_2$GP1 antigens are coated on the surface of microwells. Diluted patient serum or plasma, and calibrators, are added to the wells. The Anti ß$_2$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 IgG 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 frozenfor up to six months. Avoid repetitive freezing and thawing of serum sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1.	Microwell strips: ß2GP1 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 set (1:101 prediluted): 6.3, 12.5, 25, 50, 100, and 200 SGU.	1.0 ml /vial
7.	Control set (1:101 prediluted): Negative and Positive controls. Ranges are indicated on each label.	1.0 ml / vial
8.	Stop solution	12 ml / bottle

REAGENT PREPARATION

1. Prepare 1x washing buffer.
   Prepare washing buffer by adding distilled or deionized water to 20x wash Concentrate to make a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20- 25°C) and gently mix.

ASSAY PROCEDURE

1:101 Sample Dilution **100/100/100**
30/30/15 **RT**

1. Place the desired number of coated strips into the holder.
   PRE-WASH Coated Wells - Repeat washing three times with washing buffer.
2. Prepare 1:101 dilution of test samples by adding 5 µl of the sample to 500 µl of Sample Diluent. Mix well.
   Do not dilute 1:101 prediluted Calibrators & Controls.
3. Dispense 100 µl of diluted sera and prediluted calibrators & 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.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate into each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of Stop solution to stop reaction.
   Make sure there are no air bubbles in each well before reading.
9. Read O.D. at 450 nm with a microwell reader.

RESULTS

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

Calibrator Set	β_2GP1 IgG (SGU)	O.D. 450 nm		O.D. 450 nm Mean	SD	CV %
Calibrator 1	6.3	0.138	0.130	0.134	0.006	4.222
Calibrator 2	12.5	0.275	0.259	0.267	0.011	4.237
Calibrator 3	25	0.443	0.485	0.464	0.030	6.401
Calibrator 4	50	0.949	0.926	0.938	0.016	1.735
Calibrator 5	100	1.565	1.559	1.562	0.004	0.272
Calibrator 6	200	2.102	2.016	2.059	0.061	2.953

QUALITY CONTROL

1. 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.
2. The O.D. value of Sample diluent (0 SGU) must be lower than 0.150 and the O.D. value of calibrator 200 SGU 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 it 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: **< 20 SGU**
Low positive: **20 – 40 SGU**
Moderate positive: **40 - 70 SGU**
High positive: **> 70 SGU**

A positive result suggests the possibility of certain autoimmune disease thrombolic disorders. A negative result indicates no ß$_2$GP1 IgG antibody or levels below the detection limit of the assay.

PERFORMANCE CHARACTERISTICS

Sensitivity

The lower detection limit for ß$_2$GP1 IgG antibodies was determined at 1 SGU which corresponds to the OD that is two standard deviations from the mean OD of 20 determinations of zero concentration of anti-ß$_2$GP1 IgG antibodies.

Parallelism

In dilution experiments, sera with three different concentrations of IgG were diluted and assayed:

Sample	Dilution	Observed (SGU)	Expected (SGU)	% Recovery
Sample 1	1:100	80		
	1:200	36	40	90%
	1:400	17	20	85%
	1:800	8.5	10	85%
Sample 2	1:100	120		
	1:200	50	60	83%
	1:400	27	30	90%
	1:800	12	15	80%
Sample 3	1:100	206		
	1:200	130	103	126 %
	1:400	60	52	115%
	1:800	27	26	103%

Relative sensitivity, specificity and agreement

Comparison with a reference ß$_2$GP1 IgG kit

A total of 75 samples were assayed with the DIAGNOSTIC AUTOMATION ELISA ß2GP1 IgG (X values) and with a reference ELISA (1) (Y values). The correlation equation is

Y = 1.0327 X + 1.0057 **R^2 = 0.9815** **(n = 75)**

		Reference ELISA (1)		
		N	P	Total
Diagnostic automation ELISA ß$_2$GP1 IgG	N	49 (D)	2 (B)	51
	P	4 (C)	20 (A)	24
	Total	53	22	75

Relative sensitivity = A / (A+B) = 20 / (20 + 2) = 91 %
Relative specificity = D / (C+D) = 49 / (4 + 49) = 92 %
Agreement = (A+D) / (A+B+C+D) = (20 + 49) / (20 + 2 + 4 + 49) = 69 / 75 = 92 %

Among 4 samples which reference ELISA (1) tested for negative and DIAGNOSTIC AUTOMATION ELISA tested for positive, all 4 samples gave positive results with a second reference ELISA (2) assay.

Comparison with a reference Cardiolipin ELISA kit

A total of 77 samples were assayed with the DIAGNOSTIC AUTOMATION ELISA ß$_2$GP1 IgG (X values) and with a reference Cardiolipin (2) (Y values). The correlation equation is

Y = **1.092 X + 3.4339** R2 = **0.7954** (n = 77)

		Reference Cardiolipin (2)		
		N	P	Total
Diagnostic automation ELISA ß$_2$GP1 IgG	N	24 (D)	27 (B)	51
	P	2 (C)	24 (A)	26
	Total	26	51	77

Relative sensitivity = A / (A+B) = 24 / (24 + 27) = 47 %
Relative specificity = D / (C+D) = 24 / (2 + 24) = 92 %
Agreement = (A+D) / (A+B+C+D) = (24 + 24) / 77 = 62 %

The relative sensitivity of the IgG ß$_2$ GP1 appear to be low in comparison to those of the IgG anti- cardiolipin. It is expected because the cardiolipin as an antigen it will recognize antibodies to ß$_2$ GP1, phospholipid as well as infectious antigens, (most of these were positive syphilis).

Expected value

145 serum specimens obtained from normal, asymtomatic blood donors were tested with the DIAGNOSTIC AUTOMATION-ß$_2$ GP1 IgG test. The mean SGU =4, SD =3.

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 SGU	SD	% CV
Serum A	8	16.3	1.17	7.17
Serum B	8	33.8	1.25	3.68
Serum C	8	67.1	4.55	6.78

Inter-assay	n	Mean SGU	SD	% CV
Serum A	8	16.5	1.39	7.94
Serum B	8	35.9	2.17	6.04
Serum C	8	69.4	2.83	4.07

INTERFERENCE AND CROSS-REACTIVITY

DIAGNOSTIC AUTOMATION ß$_2$GP1 IgA test does not cross-react with the following positive samples tested: Rubella, Toxo, CMV, H. pylori, Measles, Mumps, EBV VCA, H. pylori, RF and ANA.

LIMITATIONS OF THE ASSAY

1. Diagnosis cannot be made on the basis of anti ß2 GP1 results alone. These results must be used in conjunction with information from clinical evaluation and other diagnostic procedure.
2. The clinical significance of ß2 GP1 antibodies in diseases other than SLE is currently under investigation.
3. When negative anti ß2 GP1 titers are found in the presence of clinical indications, a lupus anticoagulant, anti-cardiolipin or other additional testing is indicated.
4. It is to be expected that some samples can be anti-cardiolipin positive yet anti ß2 GP1 negative. The anti ß2 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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ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
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Date Adopted	2023-09
Brand Name	AccuDiag™
REF 1495-11	AccuDiag™- Beta 2 Glycoprotein 1 IgG ELISA
EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
Revision Date: 2016-05-25	

SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample dilution 1:10 = 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		

MANUFACTURER AND BRAND DETAILS