



DIAGNOSTIC AUTOMATION, INC.

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IVD



See external label



2°C-8°C



Σ=96 tests

REF

Cat # 1496-11

Beta 2 Glycoprotein 1 IgM

Cat # 1496-11

Cat # Number	1490Z
Test	Beta 2 Glycoprotein 1 IgM
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antigen Coated Plate
Detection Range	3.13-100SMU
Sample	5ul
Specificity	85.4%
Sensitivity	92.6%
Total Time	~90min
Shelf Life	12 months

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

NAME AND INTENDED USE

The DIAGNOSTIC AUTOMATION β_2 GP1 IgM Enzyme-linked Immunosorbent Assay (ELISA) is intended for the detection and semiquantitative 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 lupus-like disorders.

SUMMARY AND EXPLANATION OF THE TEST

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.

PRINCIPLE OF THE TEST

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.

STORAGE AND STABILITY

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.

SPECIMEN COLLECTION AND HANDLING

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 PROVIDED

- | | |
|--|--------------------|
| 1. Microwell strips: β_2 GP1 antigen coated wells. | 12 x 8 wells |
| 2. Absorbent solution: Black Cap. (For IgM test only). | 60 ml / bottle |
| 3. Washing concentrate 10x. | 100 ml / bottle |
| 4. TMB Chromogenic Substrate: Amber bottle. | 15 ml / bottle |
| 5. Enzyme conjugate: Red color solution. | 12 ml / bottle |
| 6. Calibrator Stock. 100 SMU. | 160 μ l / vial |
| 7. Control set: Negative and Positive controls.
Ranges are indicated on each label. | 160 μ l / vial |
| 8. Stop solution: 1.5 N acid solution. (HCl / H ₂ SO ₄) | 12 ml / bottle |

WARNINGS AND 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.

PREPARATION FOR ASSAY

1. Prepare 1x washing buffer.
Prepare washing buffer by adding distilled or deionized water to 10x 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.
3. Preparation of Calibrator Curve. It is recommended to use the calibrator set within 24 hours.
For Calibrator A (100 SMU), add 10 μ l of calibrator stock to 1 ml of Absorbent Solution. Prepare the Calibrators B, C, D, E and F by serial dilution of 500 μ l of Calibrator A with equal volume of Absorbent Solution.

Calibrator	Add		to Absorbent Solution	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

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 and controls by adding 5 µl of the sample to 500 µl of absorbent solution. 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 30 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.

CALCULATION OF 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	β ₂ GP1 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	13.823
Calibrator E	6.25	0.135	0.135	0.135	0.000	0.000
Calibrator D	12.5	0.255	0.254	0.255	0.001	0.278
Calibrator C	25	0.494	0.496	0.495	0.001	0.286
Calibrator B	50	0.961	0.931	0.946	0.021	2.242
Calibrator A	100	1.700	1.606	1.653	0.066	4.021

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 (Absorbent solution) 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 OF RESULTS

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 β_2 GP1 IgM antibody or levels below the detection limit of the assay.

LIMITATIONS OF THE TEST

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 IgG 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.

PERFORMANCE CHARACTERISTICS

Sensitivity, specificity, and accuracy:

A total of 75 samples were assayed with the DIAGNOSTIC AUTOMATION ELISA β_2 GP1 IgM (X values) and with a reference ELISA (1) (Y values). The correlation equation is

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

Among 19 samples which reference ELISA (1) tested for positive and DIAGNOSTIC AUTOMATION ELISA tested for negative, 12 samples were tested for negative by a second reference ELISA (2). Among 5 samples which reference ELISA (1) tested for negative and DIAGNOSTIC AUTOMATION ELISA tested for positive, 3 samples were tested for positive results by a second reference ELISA (2).

After the reconciliation, the results are summarized:

	Reference ELISA			Total
	N	P		
DIAGNOSTIC AUTOMATION ELISA β₂GP1 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 β₂GP1 IgG and IgA does not decrease the testing sensitivity for the anti β₂GP1 IgM.
2. When a sample containing a high concentration of RF IgM in the presence of a high titer of anti β₂GP1 IgG, it may induce the potential of false positive.
3. DIAGNOSTIC AUTOMATION anti β₂GP1 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.

REFERENCES

1. Hughes, G.R.V., Harris, E.N. and Gharavi, A.E. The Anticardiolipin Syndrome. The Journal of Rheumatology 1986; 13, 3: 486 – 489.
2. McNeil, H.P., R.J. Simpson, C.N. Chesteman, and S.A. Krilis. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: β₂-glycoprotein (apolipoprotein H), Proc Natl Acad Sci USA, 87: 4120 – 4124, 1990.
3. Galli, M., P. Comfurius, C. Maassen, H.C. Hemker, M.H. De Baets, P.J.C. Van Breda-Vriesman, T. Barbui, R.F.A. Zwaal, and E.M. Bevers. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a

plasma protein cofactor. Lancet, 335: 1544 – 1547, 1990.

4. Matsuura, E., Y. Igarashi, M. Fujimoto, K. Ichikawa, and T. Koike. Anticardiolipin cofactor (s) and differential diagnosis of autoimmune disease. Lancet, 336: 177 – 178, 1990.
5. Koike, T. and E. Matsuura. What is the “true” antigen for anticardiolipin antibodies? Lancet, 337: 671 – 672, 1991.
6. Matsuura, E., Y. Igarashi, M. Fujimoto, K. Ichikawa, T. Suzuki, T. Sumida, T. Yasuda, and T. Koike. Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. J Immunol, 148: 3885 – 3891, 1992.
7. Nimph, J., H. Wurm, and G.M. Kostner. β_2 -glycoprotein (apo-H) inhibits the release reaction of human platelets during ADP-induced aggregation. Atherosclerosis, 63: 109 – 114, 1987.
8. Viard, J.P., Z. Amoura, and J.F. Bach. Association of anti- β_2 -glycoprotein I antibodies with lupus-type circulating anticoagulant and thrombosis in systemic lupus erythematosus. Am J Med, 93: 181 – 186, 1992.
9. Tsutsumi A. et.al. Antibodies to β_2 -glycoprotein1 and clinical manifestations in patients with Systemic Lupus Erythematosus. Arthritis and Rheumatism, 39:1466 –1474, 1996.
10. Lee, B., Siliver, R.M. et. al. Immunoglobulin A anti- β_2 GP1 antibodies in women who experience unexplained recurrent spontaneous abortion and unexplained fetal death. Am J Obstet Gynecol, 9: 748, 2001.

Date Adopted	Reference No.
2006-06-02	DA-Beta 2 Glycoprotein 1 IgM-2009



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