

GENLISA® Beta 2 Glycoprotein IgG/IgM ELISA

REF : KBD286

Ver 2.4

IVD

ELISA immunoassay for quantitative determination of Beta 2 Glycoprotein IgG/IgM in serum and plasma.

IVD	For Invitro Diagnostic Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KBD286 **96 tests****Krishgen Pudgala LLP**Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400018. India.
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Introduction:

Beta 2 Glycoprotein 1 (B2GP1) is a 326 amino acid phospholipid binding plasma glycoprotein synthesized by hepatocytes, endothelial and trophoblast cells, and is arranged in 5 short consensus domains (I-V). Autoantibodies directed against B2GP1 (both IgG and IgM classes) are also referred to as antiphospholipid antibodies (aPLs). B2GP1 autoantibodies are part of a broader class of aPLs which also includes anti-Cardiolipin autoantibodies and Lupus Anticoagulant. Domain 1 of B2GP1 is considered to be the most clinically relevant autoantigenic part and is specifically correlated to the autoimmune disease antiphospholipid syndrome (APS). APS is an autoimmune disease characterized by recurrent vascular thrombosis (thrombotic APS) as well as by pregnancy-related complications (obstetrical APS), in combination with the presence and persistence of aPLs in patient sera. APS can occur alone (primary APS) or in association with other systemic autoimmune diseases such as systemic lupus erythematosus (secondary APS).

Intended Use:

The GENLISA® Beta 2 Glycoprotein IgG/IgM ELISA is intended for the quantitative determination of Beta 2 Glycoprotein IgG/IgM in serum and plasma.

Principle:

GENLISA® Beta 2 Glycoprotein IgG/IgM ELISA is a sandwich ELISA which is designed to quantitatively detect Beta 2 Glycoprotein IgG/IgM present in the human serum and plasma. Highly purified Beta 2 Glycoprotein is pre-coated onto microwells. Samples and Controls are pipetted into microwells and Beta 2 Glycoprotein IgG/IgM present in test sample binds to the antigen coated on the wells. And then enzyme labeled antibody conjugate is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of Anti- Beta 2 Glycoprotein IgG/IgM present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Beta 2 Glycoprotein Coated Plate (96 wells) - 1 no
2. Anti-Beta 2 Glycoprotein IgG Standards (1.2 ml/vial) - 0, 10, 20, 40, 160 AU/ml
3. Anti-Beta 2 Glycoprotein IgM Standards (1.2 ml/vial) - 0, 10, 20, 40, 160 AU/ml
4. IgG Positive Control – 1.2 ml
5. IgG Negative Control – 1.2 ml
6. IgM Positive Control – 1.2 ml
7. IgM Negative Control – 1.2 ml
8. Anti-human-IgG:HRP Conjugate – 15 ml
9. Anti-human-IgM:HRP Conjugate – 15 ml
10. Sample Diluent – 2 x 50 ml
11. (10X) Wash Buffer – 50 ml
12. TMB Substrate - 15 ml
13. Stop Solution - 15 ml
14. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipette to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

**Sample Preparation and Storage:**

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

Preparation of Samples:

All serum or plasma samples must be pre-diluted 1:100 with sample diluent; for example, 10 µl of sample should be diluted with 990 µl of sample diluent.

Reagent Preparation:

1. Allow all components to reach room temperature prior to use in the assay.
2. (1X) Wash Buffer Dilution: To make (1X) Wash Buffer, add 50 ml of (10X) Wash Buffer to 450 ml of DI water. This is the working solution.

Test Procedure:

1. Add **100 µl Diluted Sample, Standards (IgG or IgM), Positive Controls (IgG or IgM) and Negative Controls (IgG or IgM)** into appropriate wells.
2. Incubate at Room Temperature for **60 minutes**.
3. Aspirate and wash plate 3 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
4. Add **100 µl Anti-human-IgG:HRP Conjugate or Anti-human-IgM:HRP Conjugate** into Respective well. Mix well.
5. Incubate at Room Temperature for **30 minutes**.
6. Aspirate and wash plate 3 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7. Add **100 µl of TMB Substrate** to all wells.

8. Incubate at Room Temperature for 15 minutes.
9. Add **100 µl** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 5 minutes of stopping the reaction.

Calculation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Beta 2 Glycoprotein IgG/IgM concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Beta 2 Glycoprotein IgG/IgM Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a 4-PL (2nd order), or cubic spline is best recommended for automated results.

Measuring Range:

For assessment of IgG class antibodies

The assay measuring range (AMR) is 3.30 – 160 AU/mL.

Any value that reads below 3.30 AU/mL should be reported as "< 3.30 AU/mL". Any value that reads above 160 AU/mL should be reported as "> 160 AU/mL".

For assessment of IgM class antibodies

The assay measuring range (AMR) is 2.81 – 160 AU/mL.

Any value that reads below 2.81 AU/mL should be reported as "< 2.81 AU/mL". Any value that reads above 160 AU/mL should be reported as "> 160 AU/mL".

Metrology and Traceability:

For assessment of IgG class antibodies

The calibrators of this kit are traceable to the international standard for Anti-Beta 2 Glycoprotein IgG (IS 21/266)

For assessment of IgM class antibodies

Since no certified reference material is available, traceability is managed through assessment of diagnostics sensitivity and specificity.

Interpretation of Results:

Concentration	Interpretation
< 16 AU/mL	The sample should be considered negative
16 – 20 AU/mL	The sample should be graded equivocal and repeat testing / sampling should be performed according to internal practices
> 20 AU/mL	The sample should be considered Positive

Determination of a range of expected values for a "normal" population of 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.

Positive results should be verified concerning the entire clinical status of the patient, with the decision for therapy being taken on an individual basis. It is recommended that each laboratory establishes its own normal and pathological ranges of Anti-Beta 2 Glycoprotein 1 antibody values.

Performance Characteristics:

Representative performance data are shown. Results obtained at individual laboratories may vary.

Detection Capability:**For assessment of IgG class antibodies**

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 6 blanks and 6 low level samples.

Sensitivity	Concentration
Limit of Blank (LoB)	1.17 AU/ml
Limit of Detection (LoD)	2.26 AU/ml
Limit of Quantitation (LoQ)	3.30 AU/ml

For assessment of IgM class antibodies

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 6 blanks and 6 low level samples.

Sensitivity	Concentration
Limit of Blank (LoB)	1.07 AU/ml
Limit of Detection (LoD)	1.96 AU/ml
Limit of Quantitation (LoQ)	2.81 AU/ml

Trueness:**For assessment of IgG class antibodies**

Trueness has been demonstrated through a recovery test for the Beta 2 Glycoprotein for the assessment of IgG class antibodies with the International standard for Anti-β2 Glycoprotein I Immunoglobulin G (IS 21/266).

For assessment of IgM class antibodies

Since no certified reference material is available, trueness is managed through assessment of diagnostic sensitivity and specificity

Diagnostic Sensitivity and Specificity**For assessment of IgG class antibodies**

The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 50 negative and 73 positive samples run on two reagent lots.

True State	Beta 2 Glycoprotein IgG		Total
	Positive	Negative	
Positive	67	6	73
Negative	0	50	50
Total	67	56	123

Diagnostic sensitivity: 92%

Diagnostic specificity: 100%

For assessment of IgM class antibodies

The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 73 negative and 74 positive samples run on two reagent lots.

True State	Beta 2 Glycoprotein IgM		Total
	Positive	Negative	
Positive	67	7	74
Negative	0	73	73
Total	67	80	147

Diagnostic sensitivity: 90.5%

Diagnostic specificity: 100%

Precision:**For assessment of IgG class antibodies**

Precision of the Beta 2 Glycoprotein for the assessment of IgG class antibodies was determined by performing a complex precision study.

For assessment of IgM class antibodies

Precision of the Beta 2 Glycoprotein for the assessment of IgM class antibodies determined by performing a complex precision study.

Repeatability:**For assessment of IgG class antibodies**

A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.

Data from one representative lot is shown below:

Sample	n	Mean Conc. (AU/mL)	Within run (Repeatability)	
			SD	CV%
1	75	8.19	0.51	6.3%
2	75	19.96	0.78	3.9%
3	75	40.17	2.14	5.3%
4	75	71.51	3.34	4.7%
5	75	103.36	2.95	2.9%
6	75	146.46	9.85	6.7%

For assessment of IgM class antibodies

A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.

Data from one representative lot is shown below:

Sample	n	Mean Conc. (AU/mL)	Within run (Repeatability)	
			SD	CV%
1	75	9.85	0.50	5.1%
2	75	20.77	0.80	3.9%
3	75	38.67	2.26	5.8%
4	75	65.52	4.78	7.3%
5	75	88.86	3.95	4.4%
6	75	137.57	7.33	5.3%

Reproducibility:**For assessment of IgG class antibodies**

A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.

Results for the combined data from two lots is shown below:

Sample	n	Mean Conc. (AU/mL)	Within Laboratory (Reproducibility)	
			SD	CV%
1	150	8.38	0.74	8.8%
2	150	19.97	1.01	5.1%
3	150	39.06	3.75	9.6%
4	150	69.69	4.86	7.0%
5	150	99.72	7.32	7.3%
6	150	143.46	10.79	7.5%

For assessment of IgM class antibodies

A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.

Results for the combined data from two lots are shown below:

Sample	n	Mean Conc. (AU/mL)	Within Laboratory (Reproducibility)	
			SD	CV%
1	150	9.19	1.26	13.7%
2	150	20.43	1.52	7.4%
3	150	39.27	3.08	7.8%
4	150	67.48	6.33	9.4%
5	150	92.00	8.00	8.7%
6	150	140.35	10.89	7.8%

Linearity:**For assessment of IgG class antibodies**

Linearity was evaluated based on CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". For Beta 2 Glycoprotein 1 IgG concentration by Anti beta 2 Glycoprotein 1 Screen, the measurement procedure shows linearity for the interval from 3.30 to 160 AU/mL within the allowable deviation of linearity (ADL) of $\pm 15\%$.

For assessment of IgM class antibodies

Linearity was evaluated based on CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". For Beta 2 Glycoprotein 1 IgM concentration by Anti beta 2 Glycoprotein 1 Screen, the measurement procedure shows linearity for the interval from 2.81 to 160 AU/mL within the allowable deviation of linearity (ADL) of $\pm 15\%$.

Analytical Specificity:**For assessment of IgG class antibodies**

The following substances do not interfere with a bias of $>\pm 15\%$ in the Beta 2 Glycoprotein assay when assessing IgG class antibodies when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin Conjugated	15 mg/dL
Bilirubin Unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglycerides	500 mg/dL

For assessment of IgM class antibodies

The following substances do not interfere with a bias of $>\pm 15\%$ in the Beta 2 Glycoprotein assay when assessing IgM class antibodies when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin Conjugated	15 mg/dL
Bilirubin Unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglycerides	500 mg/dL

Serum-plasma study:**For assessment of IgG class antibodies**

The Beta 2 Glycoprotein matrix comparison study for assessment of IgG class antibodies was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP35-ED1 guidelines. A total of 20 samples (16 native, 4 spiked) to cover the range were evaluated. Bablok regression analysis was performed on the comparative data:

Sample Type	Slope (95% CI)	Intercept (AU/mL) (95% CI)	Correlation Coefficient (r)
SST	0.96 (0.93 to 1.00)	1.04 (-0.67 to 2.75)	1.00
Lithium Heparin	0.94 (0.88 to 1.00)	1.43 (-1.31 to 4.17)	0.99
Sodium Heparin	0.95 (0.91 to 0.99)	0.85 (-1.00 to 2.70)	1.00
EDTA	0.95 (0.91 to 0.98)	1.09 (-0.41 to 0.98)	1.00

For assessment of IgM class antibodies

The Beta 2 Glycoprotein matrix comparison study for assessment of IgM class antibodies was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP35-Ed1 guidelines. A total of 24 samples (19 native, 5 spiked) to cover the assay range were evaluated. Bablok regression analysis was performed on the comparative data:

Sample Type	Slope (95% CI)	Intercept (AU/mL) (95% CI)	Correlation Coefficient (r)
SST	0.95 (0.88 to 1.01)	1.66 (-3.03 to 6.36)	0.99
Lithium Heparin	0.96 (0.89 to 1.02)	1.45 (-3.13 to 6.02)	0.99
Sodium Heparin	0.98 (0.93 to 1.02)	0.30 (-2.77 to 3.37)	1.00
EDTA	0.94 (0.88 to 1.00)	1.57 (-2.81 to 5.94)	0.99

Safety Precautions:

- This kit is For In Vitro Diagnostic Use only. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from **human body fluids** or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
 - Do not smoke, eat or drink while handling kit material
 - Always use protective gloves
 - Never pipette material by mouth
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
 - In any case GLP should be applied with all general and individual regulations to the use of this kit.



LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Unit No.1/2, Om Sainath Commercial Complex,
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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	Add 100 ul Standards, Positive Controls and Negative Controls, Diluted Samples into appropriate wells.
2	Incubate at Room Temperature for 60 minutes.
3	Aspirate and wash plate 3 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
4	Add 100 ul Anti-human-IgG:HRP Conjugate or Anti-human-IgM:HRP Conjugate into Respective well. Mix well.
5	Incubate at Room Temperature for 30 minutes.
6	Aspirate and wash plate 3 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7	Add 100 ul of TMB Substrate to all wells.
8	Incubate on shaker at Room Temperature for 15 minutes .
9	Add 100 ul of Stop Solution . Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	Microtiter Plate (96 wells)
STD	Standards
POS CONT	Positive Control
NEG CONT	Negative Control
ENZY CONJ	Anti-human-IgG:HRP Conjugate
ENZY CONJ	Anti-human-IgM:HRP Conjugate
SAMP DIL	Sample Diluent
10X WASH BUF	(10X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature