



### AccuDiag™ Beta 2 Glycoprotein 1 IgG ELISA Kit

REF 1495

PIC AD1495YS39

IVD See External Label 2-8°C 96 Tests

#### Beta 2 Glycoprotein 1 IgG ELISA

Principle	Sandwich ELISA
Detection	Quantitative
Sample	10 µL serum/plasma
Incubation Time	105 minutes
Sensitivity	90.5%
Specificity	100%
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

Beta 2 Glycoprotein 1 IgG is a manual *in vitro* diagnostic device intended for the quantitative determination of IgG class antibodies directed against β<sub>2</sub> Glycoprotein 1 in human serum or plasma from an adult population.

#### SIGNIFICANCE AND SUMMARY

Beta 2 Glycoprotein 1 (B2GP1) is a 326 amino acid phospholipid binding plasma glycoprotein synthesised 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) (7 – 9)<sup>3</sup>. Furthermore, certain studies suggest that among aPLs, B2GP1 antibodies are the most clinically relevant markers in the diagnosis of APS (2,6,8)<sup>2,4,5</sup>.

APS is an autoimmune disease characterised by recurrent vascular thrombosis (thrombotic APS) as well as by pregnancy-related complications (obstetrical APS) (3)<sup>6</sup>, in combination with the presence and persistence of aPLs in patient sera<sup>1</sup>. APS can occur alone (primary APS) or in association with other systemic autoimmune diseases such as systemic lupus erythematosus (secondary APS).

#### ASSAY PRINCIPLE

The Beta 2 Glycoprotein 1 IgG is a two-step sandwich enzyme immunometric assay (ELISA) where patient samples, calibrators or controls are incubated on microtitre plates coated with β<sub>2</sub> Glycoprotein 1. During the incubation, antibodies present in the test sample bind to the immobilised antigen. After the incubation, the bound/free separation is performed by a simple solid phase washing.

A subsequent incubation occurs with anti-human IgG conjugated with horseradish peroxidase (HRP), which binds to the immobilised antibodies. A further wash step is performed to remove excess conjugate. Then, a chromogenic substrate solution containing TMB is dispensed into the wells which reacts with the conjugated HRP and a blue colour develops that changes into yellow when the Stop Solution (H<sub>2</sub>SO<sub>4</sub>) is added. The level of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

The concentration of IgG antibodies present in the original sample is calculated through a calibration curve.

#### SAMPLE COLLECTION AND STORAGE

The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin or potassium EDTA) samples.

Sample Storage	Duration
2 – 8°C	96 hours
Freeze/thaw cycles	3 cycles

#### REAGENTS

##### Materials provided with the test kit

- Calibrators (5 vial, 1.2 mL each)**  
Phosphate buffer 0.1M, NaN<sub>3</sub> < 0.1%, BSA 3%, human serum  
CAL0  
CAL1  
CAL2  
CAL3  
CAL4
- Controls (2 vials, 1.2 mL each, ready to use)**  
Phosphate buffer 0.1M, NaN<sub>3</sub> < 0.1%, BSA 3%, human serum  
Negative Control



Positive Control

3. **Sample Diluent (1 vial, 100 mL)**  
Phosphate buffer 0.1M, NaN<sub>3</sub> < 0.1%, BSA
4. **Conjugate (1 vial, 15 mL)**  
Anti-human IgG conjugated with peroxidase, BSA 0.1%, ProClin™ > 0.0015%
5. **Coated Microplate (1 breakable microplate)**  
Microplate coated with Beta 2-Glycoprotein 1
6. **TMB Substrate (1 vial, 15 mL)**  
H<sub>2</sub>O<sub>2</sub>-TMB (0.26 g/L) (avoid any skin contact), ProClin™ < 0.0015%
7. **Stop Solution (1 vial, 15 mL)**  
Sulphuric acid 0.15M (avoid any skin contact)
8. **10X Conc. Wash Solution (1 vial, 50 mL)**  
Phosphate buffer 0.2M pH 7.4, ProClin™ > 0.0015%

### Materials required but not provided

1. Distilled water

### Auxiliary materials and instrumentation

1. Automatic dispenser
2. Precision Pipetting Devices
3. Microplate reader (450 nm, 620-630 nm)

## REAGENT STORAGE AND STABILITY

Store the kit at 2 – 8°C in the dark.

1. The kit is stable at 2 – 8°C until the expiry date stated on the external kit label.
2. Once opened, the kit is stable at 2 – 8°C for 6 months.
3. Once opened, the calibrators are stable at 2 – 8°C for 6 months.
4. The diluted wash solution is stable for 30 days at 2 – 8°C.

Important note: open the bag containing the Coated Microplate only when it is at room temperature and close it immediately after use.

## ASSAY PROCEDURE

### 1. Preparation of Calibrators and Controls

The assay system is calibrated in relative arbitrary units. The Calibrators are ready to use and have the following concentrations:

	C <sub>0</sub>	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>
AU/mL	0	10	20	40	160

The controls are ready to use.

### 2. Preparation of the Conjugate

The conjugate is ready to use. Mix gently, for 5 minutes, on a roller mixer.

### 3. Preparation of the Wash Solution

Dilute the content of the vial “10X Conc. Wash Solution” with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio.

It is possible to observe the presence of crystals within the concentrated wash solution; in this case mix at room temperature until the complete dissolution of crystals. For greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care also to transfer crystals completely by rinsing of the bottle, then mix until crystals are completely dissolved.

### 4. Preparation of Samples

The determination of β<sub>2</sub> Glycoprotein 1 IgG can be performed in serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin, or potassium EDTA) samples.

**All serum and plasma samples must be pre-diluted with sample diluent 1:100;** for example, 10 μL of sample may be diluted with 990 μL of sample diluent.

Fasting samples are not necessary and no special sample preparations are required.

Collect blood by venipuncture into vacutainers and separate serum (after clot formation) or plasma from the cells by centrifugation.

Neither bilirubin nor hemolysis have significant effect on the procedure.

Store the sample at -20°C if the determination is not performed on the same day of the sample collection. Before using, mix gently, for 5 minutes, with a roller mixer.

### 5. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, immediately store the reagents at 2-8°C: avoiding 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<sub>0</sub>-C<sub>5</sub>), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/Controls	Blank
Calibrator C <sub>0</sub> -C <sub>4</sub>	100 μL		
Controls		100 μL	
Diluted Sample		100 μL	
Incubate 60 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 μL of diluted wash solution. <b>Important note:</b> 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. <b>Automatic washer:</b> if you use automated equipment, wash the wells at least 5 times.			
Conjugate	100 μL	100 μL	
Incubate for 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 μL of diluted wash solution. <b>Washing:</b> 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.			

## QUALITY CONTROL

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls



should be treated as unknown samples, and the results analysed with appropriate statistical methods.

The kit controls provided in the kit should be tested as unknowns and are intended to assist in assessing the validity of results obtained with each assay plate.

The mean concentration of each control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate.

Diagnostic Automation Inc. recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories<sup>7</sup>.

### CALCULATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A 4-parameter logistic (4PL) curve fit, **including Calibrator 0 is required**. A smoothed spline fit including Calibrator 0 can be used. Other curve fitting algorithms are not recommended.

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting mean absorbance on the Y-axis against concentration of analyte on the X-axis. Calibrator 0 should be included in the calibration curve. Read the mean absorbance value of each unknown sample off the curve.

In order for the assay results to be considered valid the kit calibrators and control must fall within the specifications detailed in the lot specific certificate of analysis.

If a control is out of its specified range, the associated test results are invalid and samples must be retested.

#### Conversion of units

To convert results to standard units:

$$\text{IU/mL} = \text{AU/mL} \times 0.85$$

### MEASURING RANGE

The assay measuring range (AMR) is 3.30 – 160.0 AU/mL.

Any value that reads below 3.30 AU/mL should be reported as “< 3.30 AU/mL”.

Any value that reads above 160.0 AU/mL should be reported as “> 160.0 AU/mL”.

### METROLOGY AND TRACEABILITY

The calibrators of this kit are traceable to the International Standard for  $\beta$  Glycoprotein 1 Immunoglobulin G (IS 21/266).

### EXPECTED VALUES

The following ranges were determined using the Beta 2 Glycoprotein 1 IgG and are provided for information only. The 90% reference interval for apparently healthy adults were calculated by a non-parametric method following guidance from CLSI C28-A3 “Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory”.

	n	Median (AU/mL)	Reference Interval (AU/mL)
Adults	50	<3.30	<3.30

The above ranges should be considered as guidelines only; it is recommended that each laboratory establish its own expected range based upon its own patient population.

### INTERPRETATION OF RESULTS

$\beta$ 2 Glycoprotein 1 IgG (AU/mL)	Interpretation
< 16	The sample should be considered negative
16 – 20	The sample should be graded equivocal and repeat testing / sampling should be performed according to internal practices
> 20	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 Beta 2 glycoprotein 1 antibody values.

### PERFORMANCE CHARACTERISTICS

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

#### Detection Capability

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

#### Trueness



Trueness has been demonstrated through a recovery test for the Beta 2 Glycoprotein 1 IgG assay with the International Standard for  $\beta$ 2 Glycoprotein 1 Immunoglobulin G (IS 21/266).

### Diagnostic sensitivity and specificity

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 1 IgG		Total
	Positive	Negative	
Positive	67	6	73
Negative	0	50	50
Total	67	56	123

Diagnostic sensitivity: 92%

Diagnostic specificity: 100%

### Precision

Precision of the Beta 2 Glycoprotein 1 IgG was determined by performing a complex precision study.

**Repeatability:** A total of 6 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%

**Reproducibility:** A total of 6 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%

### Linearity

Linearity was evaluated based on CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". For beta 2 Glycoprotein 1 IgG concentration by Beta 2 Glycoprotein 1 IgG, the measurement procedure shows linearity for the interval from 3.30 to 160.0 AU/mL within the allowable deviation of linearity (ADL) of  $\pm 15\%$ .

### Analytical specificity

The following substances do not interfere with a bias of  $> \pm 15\%$  in the Beta 2 Glycoprotein 1 IgG assay 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
Triglyceride	500 mg/dL

### Serum-plasma study

The Beta 2 Glycoprotein 1 IgG matrix comparison study 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) were evaluated. Passing-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 – 1.00]	1.04 [-0.67 – 2.75]	1.00
Lithium Heparin	0.94 [0.88 – 1.00]	1.43 [-1.31 – 4.17]	0.99
Sodium Heparin	0.95 [0.91 – 0.99]	0.85 [-1.00 – 2.70]	1.00
EDTA	0.95 [0.91 – 0.98]	1.09 [-0.41 – 0.98]	1.00

## LIMITATIONS OF THE ASSAY

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays<sup>8</sup>. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

## 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.
- 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.
- 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 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.

9. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
10. Maximum precision is required for reconstitution and dispensation of the reagents.
11. Samples microbiologically contaminated, highly lipemic, iceteric or haemolysed should not be used in the assay.
12. Plate readers measure vertically. Do not touch the bottom of the wells.
13. **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, deproteinisation and decontamination are effective in avoiding contamination of the conjugate; **this procedure is highly recommended when the kit is processed using analysers which are not equipped with disposable tips.**  
For this purpose, Diagnostic Automation Inc. supplies a separate decontamination reagent for cleaning needles.
14. Fresh disposable tips must be used when pipetting assay reagents including samples, calibrators and controls to mitigate the risk of carryover contamination. Failure to do so may lead to invalid results.

### WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

All materials that have come into contact with samples and reagents must be disposed of in accordance with country, state and local regulations.

### WARNINGS

1. This kit is intended for *in vitro* use by professional persons only. Not for internal or external use in Humans or Animals.
2. Use appropriate personal protective equipment while working with the reagents provided.
3. Follow Good Laboratory Practice (GLP) for handling blood products.
4. 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.
5. Some reagents (conjugate and wash solution) contain small amounts of ProClin™ 300 (>0.0015%) as preservative. Avoid contact with skin or mucosa.
6. Classification according to Regulation (EC) No. 1272/2008 [CLP]

Skin sensitivity, Category 1



Contains: ProClin 300

Warning

#### Hazard statements

H317: May cause an allergic skin reaction

#### Precautionary statements

P261: Avoid breathing dust / fumes / gas / mist / vapours / spray.

P280: Wear protective gloves / protective clothing / eye protection / face protection / hearing protection.

P321: Specific treatment (see supplemental first aid instruction on this label)

P333+P313: If skin irritation or rash occurs: get medical advice/attention.

P362+P364 – Take off contaminated clothing and wash it before reuse.

7. Some reagents (calibrators, controls and sample diluent) contain small amounts of Sodium Azide (NaN<sub>3</sub>) <0.1%. 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, wash through large with amounts of water to prevent azide build-up.
8. The TMB Substrate contains an irritant, which harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
9. The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous, corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
10. Avoid the exposure of reagent TMB/H<sub>2</sub>O<sub>2</sub> to direct sunlight, metals or oxidants.

### REFERENCES

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### MANUFACTURER AND BRAND DETAILS

ISO 13485:2016



ISO 13485  
Quality  
Management for  
Medical Devices  
CERTIFIED

 Diagnostic Automation/Cortez Diagnostics, Inc.  
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Brand Name	AccuDiag™
REF 1495	AccuDiag™ - Beta 2 Glycoprotein 1 IgG ELISA
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