Anti-ß$_2$ GP-I (Beta 2 Glycoprotein 1) ELISA

Catalog Number:
B2G31-K01

Enzyme immunoassay for the determination of IgG or IgM antibodies to ß$_2$ glycoprotein-I in human plasma and serum

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v. 1.0

INTENDED USE

The Eagle Biosciences Anti-ß$_2$ GP-I ELISA Assay Kit is used for the quantitative determination of IgG or IgM antibodies to ß$_2$ glycoprotein-I (ß$_2$ GP-I) in human serum or plasma for the diagnosis of anti-phospholipid antibody syndrome (APAS).

APAS is an autoimmune disorder comprising such clinical symptoms like arterial or venous thrombosis, thrombocytopenia and recurrent fetal loss. Primary APAS as well as systemic lupus erythematosus (SLE) are characterized by the appearance of autoantibodies to negatively charged phospholipids (1). Although significance and pathological relevance of phospholipid antibodies are not completely revealed yet, the detection of several autoantibody specificities is usually applied to the differential diagnosis and follow-up of systemic rheumatic inflammatory diseases.

Unlike phospholipid antibodies which occur in some patients having infectious disease, phospholipid antibodies of autoimmune disease patients seem to recognize the relevant phospholipids in association with a plasma protein cofactor.
One of these cofactors has been identified as β_2 glycoprotein-I (β_2 GP-I) (apolipoprotein H) (2,3). β_2 GP-I, a serum protein with a molecular weight of 50 kDa affects platelet aggregation and coagulation.

The positively charged fifth domain of β_2 GP-I interacts with negatively charged phospholipids or activated polystyrol surfaces of ELISA wells. This interaction results in conformational changes of β_2 GP-I and the creation of new epitopes apparently recognized by autoimmune phospholipid autoantibodies.

(1) Harris EN, Gharavi AE, Boey ML, Patel BM, Mackworth-Young GG, Loizou S and Hughes GRV: Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 1983 11:1211
(2) Galli M, Comfurius P, Maassen C, Hemker HC, DeBaets MHVan Breda-Vriesman PJC, Barbui T, Zwaal RFA, Bevers EM: Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein factor. Lancet 1990 335:1544-1547
(3) McNeil HP, Simpson RJ, Chesterman CN, Krilis SA: Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding factor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA 1990 87:4120-4124

PRINCIPLE OF THE TEST

The Eagle Biosciences Anti- ß$_2$ GP-I ELISA Assay Kit is an enzyme immunoassay for the quantitative determination of IgG or IgM antibodies to ß$_2$ glycoprotein-I in human serum or plasma.

The antibodies of the standards, the positive control and diluted patient samples react with the human ß$_2$ GP-I, immobilized on the solid phase of microtiter plates. The use of highly purified ß$_2$ GP-I guarantees the specific binding of antibodies to ß$_2$ glycoprotein-I of the specimen under investigation. Following an incubation period of 60 min at room temperature (RT), unbound serum components are removed by a wash step.

The bound antibodies react specifically with anti-human-IgG or IgM conjugated to horseradish peroxidase (HRP) within the next incubation period of 30 min at RT. Excessive conjugate is separated from the solid-phase immune complexes by an additional wash step.

HRP converts the colorless substrate solution of 3,3`,5,5`-tetramethyl-benzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an added acidic solution (H_2SO_4) into the wells, after 15 min at RT turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specifically bound antibodies.

The standard curve is established by plotting the antibody concentrations of the standards (x-axis) and their corresponding OD-values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Specimen can be stored at 2 to 8 °C until 3 days. Long-term storage requires - 20 °C. Repeated freezing and thawing should be avoided. If necessary samples have to aliquot before freezing.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: **Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1.0 sample diluent (C), prior to assay.**

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate** ,12 breakable strips per 8 wells (total 96 individual wells) coated with highly purified human β_2 GP-I	1 vacuum-sealed with desiccant
B BUF WASH	**Wash buffer, 10 fold** sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ G	**IgG conjugate** containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped red
E CONJ M	**IgM conjugate** containing anti-human-IgM- (sheep) coupled with HRP	15 ml ready for use capped green
F SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
G H2SO4	**Stop solution** 0.25 M sulfuric acid 0.25M	15 ml ready for use capped yellow
0 - 4 CAL	**Calibrators** (diluted sera) conc.: 1, 10, 30, 100, 300 U/ml	1 ml each ready for use capped white
P CONTROL	**Positive control** (diluted serum) conc.: see leaflet enclosed +	1 ml ready for use capped red
N CONTROL	**Negative control** (diluted serum) conc.: see leaflet enclosed -	1 ml ready for use capped green

Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
  trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm

Size and storage

The Eagle Biosciences Anti-ß$_2$ GP-I ELISA Assay Kit has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Anti-ß$_2$ GP-I have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells.

Allow the sealed microplate to reach room temperature before opening.
Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash buffer by diluting the concentrated wash solution 10 times (1 + 9 v/v) with de-ionized or distilled water. For example, dilute 6 ml of the concentrate with 36 ml of distilled water per strip. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1 + 100 (v/v) e.g. 10 µl Serum + 1.0 ml sample diluent (C).**
- **Please attend the stepwise dispensing sequence and keep timing of each step.**

1. Bring all reagents to room temperature (20…25°C) before use. Mix gently without causing foam.
2. Dispense **100 µl** calibrators (0 optional) 1 - 4 (quantitative) or **100 µl** calibrator 1 (semi-quantitative)**100 µl** control P (N optional) **100 µl** diluted patient samples into the respective wells.
3. Incubate **60 min** at room temperature (20…25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D or E) solution to each well.
6. Incubate **30 min** at room temperature (20…25°C).
7. Decant, then wash each well **three** times using **300 µl**
   wash solution (made of B).
8. Add **100 µl** of substrate (F) to each well.
9. Incubate **15 min protected from light** at room temperature (20…25°C).
10. Add **100 µl** of stop solution (G) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within 30 min after adding the stop solution.

DATA PROCESSING

The Eagle Biosciences Anti-ß$_2$ GP-I ELISA Assay Kit allows both the quantitative (4 + 1 calibrators) and semi-quantitative (calibrator 1 for cut-off determination) evaluation of the results.

Quantitative evaluation

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective anti-ß$_2$ GP-I concentrations on the abscissa, x-axis, (log. scale). Anti-ß$_2$ GP-I concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Semi-quantitative evaluation

Results are interpreted by calculating the binding index (BI) using **calibrator 1 (10 U/ml)** as **cut-off calibrator**. The BI is the ratio of the OD-value of a sample to the cut-off OD-value (CAL 1).

BI = OD_{sample} / ($OD_{calibrator\ 1}$)

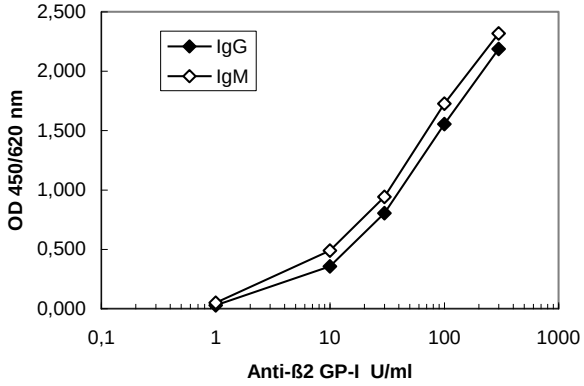
Both evaluation variants of Anti-ß$_2$ GP-I may be achieved also with computer assisted analysis software integrated in the photometers.

Example of Typical Assay Results

IgG	OD 1	OD 2	mean OD	U/ml
Calibrator **0**	0.028	0.026	0.027	**1**
Calibrator **1**	0.353	0.367	0.355	**10**
Calibrator **2**	0.871	0.816	0.804	**30**
Calibrator **3**	1.541	1.567	1.554	**100**
Calibrator **4**	2.175	2.199	2.187	**300**
Patient **1**	0.661	0.677	0.669	**23**

IgM	OD 1	OD 2	mean OD	U/ml
Calibrator **0**	0.048	0.053	0.050	**1**
Calibrator **1**	0.481	0.497	0.489	**10**
Calibrator **2**	0.936	0.948	0.942	**30**
Calibrator **3**	1.712	1.739	1.725	**100**
Calibrator **4**	2.302	2.334	2.318	**300**
Patient **1**	1.061	1.077	1.069	**36**

TYPICAL STANDARD CURVES



Test validity

The test run is valid if:

- the mean OD of the calibrator 4 is ≥ 1.2
- Concentration of Control P see leaflet enclosed
- Control N is negative

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

Anti-ß$_2$ GP-I	U/ml	BI
positive	**≥ 10**	**≥ 1,0**
negative	**< 10**	**< 1,0**

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum Anti-ß$_2$ GP-I levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the Anti-ß$_2$ GP-I. However, anti-ß$_2$ GP-I antibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are suggested to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

Due to the lack of an international reference material the Anti-ß$_2$ GP-I is calibrated in arbitrary units (U/ml).

Linearity

Dilutions of selected positive specimens in anti-ß$_2$ GP-I autoantibody free human serum are determined according to their expected theoretical values with Anti-ß$_2$ GP-I.

Sensitivity

The analytical sensitivity of the Anti-ß$_2$ GP-I is 1 U/ml for both IgG and IgM detremination.

Precision

Intra-Assay (n = 20)

	IgG		IgM	
Probe	**U/ml**	**C.V. (%)**	**U/ml**	**C.V. (%)**
Serum A	48,1	5,1	142,3	6,8
Serum B	13,3	3,7	58,1	5,1
Serum C	5,5	3,2	9,2	3,9

Inter-Assay (n = 5 x 10)

	IgG		IgM	
Probe	**U/ml**	**C.V. (%)**	**U/ml**	**C.V. (%)**
Serum A	51,0	3,6	160,5	9,6
Serum B	14,7	5,9	61,0	2,1
Serum C	6,4	7,1	10,0	4,2

ASSAY SCHEME

Dilute patients sample 10 µl serum + 1.0 ml sample diluent (C)

1	Bring all ready for use reagents to room temperature (20…25°C) before use.				
			calibrators	control	sera
2	Pipette	calibrators (0 - 4) or calibrator 1 controls (P, N) 1 + 100 prediluted patient sera	100 µl	100 µl	100 µl
3	Incubate		60 minutes at room temperature (20…25°C)		
4	Wash		Decant, 3 x 300 µl (made of B)		
5	Pipette conjugate (D or E)		100 µl	100 µl	100 µl
6	Incubate		30 minutes at room temperature (20…25°C)		
7	Wash		Decant, 3 x 300 µl (made of B)		
8	Pipette substrate (F)		100 µl	100 µl	100 µl
9	Incubate protected from light		15 minutes at room temperature (20…25°C)		
10	Pipette stop solution (G)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm within 30 min.				

SAFETY PRECAUTIONS

- **This Anti-β GP-I ELISA Assay kit is for research use only**. Follow the working instructions carefully. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted 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 at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Neolone M10 (≤ 1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials 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.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.