



INSTRUCTION MANUAL

REF 4036

April 01, 2014

Anti-β₂ GP-I Screen

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG, IgM and IgA antibodies to β₂ glycoprotein-I in human serum or plasma

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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INTENDED USE

Anti-β₂ GP-I Screen is used for the semi-quantitative determination of IgG, IgM and IgA antibodies (screening) to β₂ glycoprotein-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 β₂ glycoprotein-I (β₂ GP-I) (apolipoprotein H) (2,3). β₂ GP-I, a serum protein with a molecular weight of 50 kDa affects platelet aggregation and coagulation.

The positively charged fifth domain of β₂ GP-I interacts with negatively charged phospholipids or activated polystyrol surfaces of ELISA wells. This interaction results in conformational changes of β₂ 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

Anti-β₂ GP-I Screen is used for the semi-quantitative determination of IgG, IgM and IgA antibodies to β₂ glycoprotein-I in human serum or plasma.

The antibodies of the controls and the diluted patient samples react with human β₂ GP-I, immobilized on the solid phase of microtiter plates. The use of highly purified β₂ GP-I guarantees the specific binding of antibodies to β₂ glycoprotein-I of the specimen under investigation. Following an incubation period of 60 min at room temperature, unbound serum components are removed by a wash step.

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

HRP converts the colourless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H₂SO₄) 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 specific antibodies bound. The OD values of the unknown patient samples are compared to the OD values of the calibrator.

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.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

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 ml 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	Microtiter plate, 12 breakable strips (total 96 wells) coated with human β_2 glycoprotein-I	1	vacuum sealed with desiccant, 2 adhesive foils
Ag 96			
B	Concentrated wash buffer sufficient for 1000 ml solution	100 ml	concentrate capped white
BUF WASH	10x		
C	Sample diluent	100 ml	ready for use capped black
DIL			
D	Conjugate containing anti-human-IgGAM (goat) coupled with HPR	15 ml	ready for use capped white
CONJ			
E	Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml	ready for use capped blue
SOLN TMB			
F	Stop solution 0.25 M sulfuric acid	15 ml	ready for use capped yellow
H2SO4	0.25M		
P	Positive control (diluted serum)	1 ml	ready for use capped red
CONTROL	+		
CO	Cut-off control (diluted serum)	1 ml	ready for use capped white
CONTROL	C		
N	Negative control (diluted serum)	1 ml	ready for use capped green
CONTROL	-		

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
- distilled or de-ionized water

Size and storage

Anti- β_2 GP-I Screen has been designed for 96 determinations.

Upon receipt, all components of the Anti- β_2 GP-I Screen 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 solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 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).
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
100 µl controls (P, CO, N)
100 µl diluted patient samples
into the respective wells.
3. Incubate **60 min** at room temperature (18-25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Incubate **30 min** at room temperature (18-25°C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min** protected from light at room temperature (18-25°C).
10. Add **100 µl** of stop solution (F) 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

Results are interpreted qualitatively or semi-quantitatively by calculating the binding index (BI) for each sample on the basis of the OD of the cut-off control:

For the calculation of the binding index (ratio) the following formula should be applied:

$$BI = OD_{\text{sample}} / OD_{\text{cut-off control}}$$

This calculation can be performed by the integrated evaluation software of most microplate readers used, too.

Example of Typical Assay Results

well	OD (a)	OD (b)	OD (mean)	BI
Positive control	1.558	1.510	1.534	
Cut-off control	0.521	0.530	0.526	
Negative control	0.089	0.092	0.091	
Patient 1	1.604	1.599	1.602	3.1 - pos
Patient 2	1.261	1.287	1.274	2.4 - pos
Patient 3	0.170	0.157	0.164	0.3 - neg

Test validity

The test run is valid if:

- the mean OD of the positive control ≥ 0.6
- the mean OD of the cut-off control > mean OD of the negative control

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- β_2 GP-I	BI ratio
negative	< 1,0
positive	$\geq 1,0$

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti- β_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- β_2 GP-I Screen. However, β_2 GP-I autoantibody positive apparently healthy persons do occur.

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

CHARACTERISTIC ASSAY DATA

Calibration

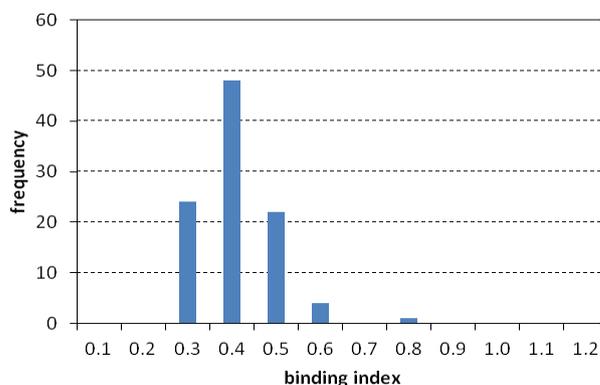
Due to the lack of international reference materials results are interpreted by calculating a BI (ratio).

Sensitivity

The analytical sensitivity of the Anti- β_2 GP-I Screen is 0.1 (BI).

Specificity

Frequency distribution of antibodies in Anti- β_2 GP-I Screen. 99 unselected human sera were tested. All sera were found negative. This corresponds to a diagnostic specificity of 100%.



Precision

The intra-assay coefficient of variation (CV) was determined by 20 fold measurements.

Intra-Assay n = 20		
mean BI	SD	CV %
13.3	0.041	2.3
7.1	0.048	3.3
2.4	0.038	4.4

The inter-assay coefficient of variation (CV) was determined by 10fold measurements in five independent runs.

Intra-Assay n = 5 x 10		
mean BI	SD	CV %
14.3	0.031	4.3
6.7	0.016	3.5
2.1	0.020	5.4

INCUBATION SCHEME

Anti- β_2 GP-I Screen (4036)

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

1	Bring all test reagents to room temperature (18...25°C).					
2	Dispense	positive control (P) cut-off control (CO) negative control (N) 1 + 100 diluted patient sera	100 μ l	100 μ l	100 μ l	100 μ l
3	Incubate 60 min, room temperature (18...25°C)					
4	Wash Decant, 3 x 300 μl (made of B)					
5	Dispense conjugate (D)		100 μ l	100 μ l	100 μ l	100 μ l
6	Incubate 30 min, room temperature (18...25°C)					
7	Wash Decant, 3 x 300 μl (made of B)					
8	Dispense substrate (E)		100 μ l	100 μ l	100 μ l	100 μ l
9	Incubate in the dark 15 min, room temperature (18...25°C)					
10	Dispense stop solution (F)		100 μ l	100 μ l	100 μ l	100 μ l
11	Read at 450 against 620 (690) nm					

SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. 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.
- All reagents should be kept at 2 - 8 °C in the original shipping container before use.
- Some of the reagents contain small amounts of Neolone M10 (< 1 % v/v) as preservatives. 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 for HIV as well as 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.