Anti-β_2 GP-I Screen ELISA

Catalog Number:
B2S31-K01

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

20A Northwest Blvd., Suite 112,
Nashua, NH 03060
Phone: 617-419-2019
Fax: 617-419-1110
www.EagleBio.com

For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
v. 1.0

INTENDED USE

The Eagle Biosciences Anti-β_2 GP-I Screen ELISA Assay Kit is used for the semi-quantitative determination of IgG, IgM and IgA antibodies (screening) to ß$_2$ 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 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--β_2 GP-I Screen ELISA Assay Kit is used for the semi-quantitative determination of IgG, IgM and IgA antibodies to ß$_2$ glycoprotein-I in human serum or plasma.

The antibodies of the controls and the diluted patient samples react with human ß$_2$ GP-I, immobilized on the solid phase of microtiter plates. The use of highly purified β_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, 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'-tetramethyl-benzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an 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 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 Ag 96	**Microtiter plate**, 12 breakable strips (total 96 wells) coated with human ß$_2$ glycoprotein-I	1 vacuum sealed with desiccant, 2 adhesive foils
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgGAM (goat) coupled with HPR	15 ml ready for use capped white
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 M sulfuric acid 0.25M	15 ml ready for use capped yellow
P CONTROL	**Positive control** (diluted serum) +	1 ml ready for use capped red
CO CONTROL	**Cut-off control** (diluted serum) C	1 ml ready for use capped white
N CONTROL	**Negative control** (diluted serum) –	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
- distilled or de-ionized water

Size and storage

Anti-ß$_2$ GP-I Screen has been designed for 96 determinations.

Upon receipt, all components of the Anti-ß$_2$ 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:

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

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- ß$_2$ GP-I	BI ratio
negative	**< 1,0**
positive	**≥ 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 Screen. However, ß$_2$ 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

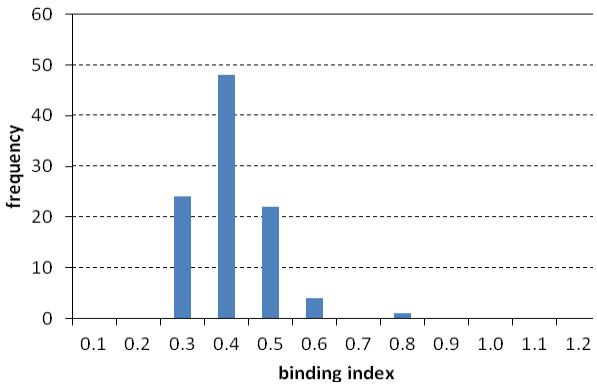
Due to the lack of international reference materials results are interpreted by calculating a BI (ratio).

Sensitivity

The analytical sensitivity of the Anti- ß$_2$ GP-I Screen is 0.1 (BI).

Specificity

Frequency distribution of antibodies in Anti- ß$_2$ 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

Anti-β_2 GP-I Screen ELISA Assay Kit

ASSAY SCHEME

Dilute patients sample **10 µl 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 µl	100 µl	100 µl	100 µl
3	Incubate		60 min, room temperature (18…25°C)			
4	Wash		Decant, 3 x 300 µl (made of B)			
5	Dispense conjugate (D)		100 µl	100 µl	100 µl	100 µl
6	Incubate		30 min, room temperature (18…25°C)			
7	Wash		Decant, 3 x 300 µl (made of B)			
8	Dispense substrate (E)		100 µl	100 µl	100 µl	100 µl
9	Incubate in the dark		15 min, room temperature (18…25°C)			
10	Dispense stop solution (F)		100 µl	100 µl	100 µl	100 µl
11	Read at 450 against 620 (690) nm					

SAFETY PRECAUTIONS

- **This Anti-β_2 GP-I Screen 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.
- 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 Anti-β_2 GP-I Screen ELISA Assay Kit 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.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

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.