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Instruction for use  
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## ORG 521S Anti-beta-2-Glycoprotein I Screen

Immunometric Enzyme Immunoassay for the semi-quantitative screening of anti- $\beta_2$ -Glycoprotein I antibodies of IgG, IgM and IgA class

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### NAME AND INTENDED USE

Anti-beta-2-Glycoprotein I Screen is an indirect solid phase enzyme immunoassay (ELISA) for the semiquantitative screening of IgG, IgM and IgA class autoantibodies against  $\beta_2$ -Glycoprotein I in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of an increased risk of thrombosis in patients with Systemic Lupus Erythematosus (SLE) or lupus-like disorders.

### SUMMARY AND EXPLANATION OF THE TEST

Anti- $\beta_2$ -Glycoprotein I antibodies are associated with the diseases of the antiphospholipid syndrome, like thrombosis, thrombocytopenia or fetal loss in the context of systemic lupus erythematosus.

$\beta_2$ -Glycoprotein I (apolipoprotein H) is a 50 kDa  $\beta_2$ -globulin occurring in plasma at a level of 200  $\mu\text{g/ml}$ . It has been found that  $\beta_2$ -Glycoprotein I ( $\beta_2\text{GPI}$ ) inhibits the intrinsic coagulation pathway and, therefore, it is involved in the regulation of blood coagulation.  $\beta_2\text{GPI}$  is associated in vivo with negatively-charged substances, e.g. anionic phospholipids, heparin and lipoproteins. The phospholipid binding region is located on its fifth domain.

Recently,  $\beta_2$ -Glycoprotein I has become well-known as a co-factor for anti-Cardiolipin autoantibodies. Several studies confirmed its indispensable role in proper binding of anti-Cardiolipin antibodies to immobilized Cardiolipin. Detailed investigations about the nature of the Cardiolipin- $\beta_2\text{GPI}$ -complex have shown that epitopes on the fifth domain of  $\beta_2\text{GPI}$  are the real target of "anti-Cardiolipin antibodies" - even in the absence of negatively-charged phospholipids.  $\beta_2\text{GPI}$  is not only a prerequisite for the binding of anti-Cardiolipin antibodies; it has now been identified as the primary antigen for these antibodies.

Samples from clinical patients with the antiphospholipid syndrome were tested for anti-Cardiolipin and anti- $\beta_2\text{GPI}$  antibodies. Good correlations between the anti-Cardiolipin and anti- $\beta_2\text{GPI}$  values confirm the statement above.

Autoantibodies against  $\beta_2$ -Glycoprotein I are described for various autoimmune diseases. The presence of anti- $\beta_2\text{GPI}$  antibodies can be related to the development of arterial and venous thromboses, venous thromboembolism, thrombocytopenia and fetal loss.

Anti- $\beta_2$ -Glycoprotein I antibodies are found in the immunoglobulin classes IgG, IgM and IgA. The determination of IgM antibodies is a valuable indicator in the diagnosis of beginning autoimmune diseases, whereas IgG antibodies will be found in progressive stages of manifested autoimmune disorders. Anti- $\beta_2\text{GPI}$  IgG antibody titers correlate well with the clinical status of the patients in thrombosis, thromboembolism and repeated fetal loss, while anti- $\beta_2\text{GPI}$  IgM antibodies show a significant association with thrombosis and thrombocytopenia.

Indications for determination of anti- $\beta_2$ -Glycoprotein I antibodies:

- SLE
- arterial and venous thromboses
- venous thromboembolism
- thrombocytopenia
- fetal loss

## PRINCIPLE OF THE TEST

Highly purified  $\beta$ 2-glycoprotein I is bound to microwells. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG, IgM and IgA immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG, IgM, IgA antibodies present in the original sample.

## WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 and HIV2 and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is hydrochloric acid (1 M). If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide ( $\text{NaN}_3$ ) is highly toxic and reactive in pure form. At the product concentrations (0,09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

## CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified $\beta$ 2-Glycoprotein I. Ready to use.
4 vials, 1.5 ml each	controls with Anti- $\beta$ 2-Glycoprotein I antibodies of IgG/IgM/IgA class in serum/buffer matrix (PBS, BSA, $\text{NaN}_3$ <0,1% (w/w)). Ready to use. Negative Control (A) 3.3 U/ml Cut-Off Control (B) 10 U/ml Positive Control (C) 30 U/ml Strong positive Control (D) 90 U/ml
1 vial, 20 ml	Sample buffer (Tris, $\text{NaN}_3$ <0,1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	Enzyme conjugate solution (PBS, PROCLIN 300 <0,5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG, polyclonal rabbit anti-human IgM and polyclonal rabbit anti-human IgA; each labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.
1 vial, 15 ml	Stop solution (1 M hydrochloric acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, $\text{NaN}_3$ <0,1% (w/w)), concentrate (50x).

## STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

## MATERIALS REQUIRED

### Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100  $\mu$ l
- Vortex mixer
- Pipets for 10  $\mu$ l, 100  $\mu$ l and 1000  $\mu$ l
- Laboratory timing device
- Data reduction software

### Preparation of reagents

- Distilled or deionized water

- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

### SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

### PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

### PREPARATION OF REAGENTS

#### Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

#### Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

#### Sample preparation

Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

### TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of controls and prediluted patient samples in duplicate into the wells.

	1	2	3	4	5	6
A	CA	P1	P..			
B	CA	P1	P..			
C	CB	P2				
D	CB	P2				
E	CC	P3				
F	CC	P3				
G	CD	P4				
H	CD	P4				

CA - CD: Controls A to D  
P1, P2... patient sample 1, 2 ...

3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

## Automation

The ORGENTEC Anti-beta-2-Glycoprotein I Screen ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

## INTERPRETATION OF RESULTS

### Quality Control

This test is only valid if the optical density at 450 nm for Negative Control (A), Cut-Off Control (B), Positive Control (C) and Strong Positive Control (D) complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

### Qualitative evaluation of ELISA

Evaluation of the Anti-beta-2-Glycoprotein I Screen test is carried out by direct comparison of the optical density of each patient sample with the optical density of the controls.

Patient samples exhibiting optical densities higher than the optical density of the cut-off control are considered to be positive.

Negative:	OD Patient	<	OD Cut-Off Control
Positive:	OD Patient	>	OD Cut-Off Control
Strong Positive:	OD Patient	≥	OD Strong Positive Control

### Quantitative evaluation of ELISA

For quantitative calculation of the patients results the concentration of the controls may be used for creating a calibration curve. For Anti-beta-2-Glycoprotein I Screen a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is recommended. The concentration of unknowns may be calculated from this calibration curve.

Cut-off: 10 U/ml

Further differentiation and typing should be carried out using the fully quantitative Anti-beta-2-Glycoprotein I IgG/IgM and/or IgA kits. The Anti-beta-2-Glycoprotein I Screen recognises the sum of IgG, IgM and IgA class anti-β2-Glycoprotein I autoantibodies. Due to additive effects, patient samples containing two or three Anti-β2-Glycoprotein I antibody classes with positive results in the Anti-beta-2-Glycoprotein I screen may be determined as negative using the single Anti-beta-2-Glycoprotein I IgG/ IgM or IgA assays.

## PERFORMANCE CHARACTERISTICS

### Specificity

The microplate is coated with highly purified human β2-Glycoprotein I. The test kit is specific only for autoantibodies against β2-Glycoprotein I. Endogenous β2-Glycoprotein I and endo-

genous negatively-charged phospholipids occur in (1:100)-diluted samples at approx. 2 µg/ml and approx. 1 µg/ml, respectively. Influences on the determination of anti-β2-Glycoprotein I-antibodies have not been observed.

### Calibration

Since no international reference preparation for anti-β2-Glycoprotein I autoantibodies is available, the assay system is calibrated in relative arbitrary units. The calibration is related to the internationally recognized reference sera from E.N. Harris, Louisville. These sera test positive for anti-β2-Glycoprotein I autoantibodies.

### LIMITATIONS OF PROCEDURE

The Anti-beta-2-Glycoprotein I Screen ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

### INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

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#### INCUBATION SCHEME

