DRG® Anti-β2 Glycoprotein I IgG/IgM (EIA-3590)

Revised 1 Dec. 2009 (Vers. 2.0) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

NAME AND INTENDED USE

Anti-beta-2-Glycoprotein I IgG/IgM is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG and IgM class autoantibodies against β2-Glycoprotein I in human serum or plasma. The assay is intended for in vitro use only as an aid in the determination of an increased risk of thrombosis in patients with Systemic Lupus Erythematosus (SLE) or lupus-like disorders.

SUMMARY AND EXPLANATION OF THE TEST

Anti-β2-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. The Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis has issued consensus criteria that may be used to help laboratory diagnosis. These criteria have been updated in 2006.
β2-Glycoprotein I (apolipoprotein H) is a 50 kDa β2-globulin occurring in plasma at a level of 200 µg/ml. It has been found that β2-Glycoprotein I (β2GPI) inhibits the intrinsic coagulation pathway and, therefore, it is involved in the regulation of blood coagulation. β2GPI 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, β2-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-β2GPI-complex have shown that epitopes on the fifth domain of β2GPI are the real target of "anti-Cardiolipin antibodies" - even in the absence of negatively-charged phospholipids. β2GPI 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-β2GPI antibodies. Good correlations between the anti-Cardiolipin and anti-β2GPI values confirm the statement above.
Autoantibodies against β2-Glycoprotein I are described for various autoimmune diseases. The presence of anti-β2GPI antibodies can be related to the development of arterial and venous thromboses, venous thromboembolism, thrombocytopenia and fetal loss.
Anti-β2-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-β2GPI IgG antibody titers correlate well with the clinical status of the patients in thrombosis, thromboembolism and repeated fetal loss, while anti-β2GPI IgM antibodies show a significant association with thrombosis and thrombocytopenia.
Indications for determination of anti-β2-Glycoprotein I antibodies:

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

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PRINCIPLE OF THE TEST

Highly purified β2-glycoprotein I is bound to microwells. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigens. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG and IgM 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 resp. IgM antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro 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 or 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 acid. 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 (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 β2-Glycoprotein I. Ready to use.
6 vials, 1.5 ml each	combined Calibrators with IgG and IgM class Anti-β2-glycoprotein I antibodies (A-F) in a serum/buffer matrix (PBS,BSA,NaN_3 <0,1% (w/w)) :

	IgG: 0; 6.3; 12.5; 25; 50; and 100 U/ml and IgM: 0; 6.3; 12.5; 25; 50; 100 U/ml. Ready to use.
2 vials, 1,5 ml each	Anti-β2-glycoprotein I Controls in a serum/buffer matrix (PBS, BSA,NaN_3 <0,1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed QC insert. Ready to use.
1 vial, 20 ml	Sample buffer (Tris, 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; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	Enzyme conjugate solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human-IgM; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.
1 vial, 15 ml	Stop solution (contains acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, 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 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- 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 semiquantitatively.

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 1**00 µl** of calibrators, controls and prediluted patient samples in duplicate into the wells.

	1	*2*	*3*	*4*	*5*	*6*
A	**SA**	**SE**	**P1**	**P5**		
B	**SA**	**SE**	**P1**	**P5**		
C	**SB**	**SF**	**P2**	**P..**		
D	**SB**	**SF**	**P2**	**P..**		
E	**SC**	**C1**	**P3**			
F	**SC**	**C1**	**P3**			
G	**SD**	**C2**	**P4**			
H	**SD**	**C2**	**P4**			

SA - SF: standards A to F
P1, P2... patient sample 1, 2 ...
C1: positive control
C2: negative control

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 (Anti-h-IgG or Anti-h-IgM) 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 Anti-beta-2-Glycoprotein I IgG/IgM 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 Positive Control (1) and Negative Control (2) as well as for the Standards A and F 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.

Calculation of results

For the Anti-beta-2-Glycoprotein I IgG/IgM test a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calculation Example

The figures below show typical results for Anti-beta-2-Glycoprotein I IgG/IgM ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

Calibrators									
No	**Position**	**OD 1**	**OD 2**	**Mean**	**Conc. 1**	**Conc. 2**	**Mean**	**decl. Conc.**	**CV %**
STA	A 1/B 1	0.124	0.122	0.123	0.001	0.001	0.001	0.001	1
STB	C 1/D 1	0.281	0.282	0.281	5.4	5.4	5.4	6.3	0
STC	E 1/F 1	0.579	0.578	0.579	12.9	12.9	12.9	12.5	0
STD	G 1/H 1	0.990	0.995	0.993	25.2	25.4	25.3	25.0	1
STE	A 2/B 2	1.463	1.488	1.476	48.6	50.5	49.5	50.0	1
STF	C 2/D 2	1.862	1.865	1.863	99.7	100.4	100.1	100.0	0

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-beta-2-Glycoprotein I IgG/IgMtests:

	anti-β2-Glycoprotein I Ab	
	IgG [U/ml]	**IgM [U/ml]**
normal:	< 5	< 5
borderline:	5 - 8	5 - 8
elevated:	> 8	> 8

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.
It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-β2-glycoprotein I. The values below should be regarded as guidelines only.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high IgG- and IgM-antibody concentrations were diluted with sample buffer and assayed in the Anti-beta-2-Glycoprotein I IgG/IgM kit.

Anti-β2-GPI	Sample	Dilution	Observed [U/ml]	Expected [U/ml]	O/E
IgG	1	1:200	100.0		
		1:400	49,8	50.0	100%
		1:800	25.5	25.0	102%
		1:1600	13.1	12.5	105%
		1:3200	6.9	6.3	110%
		1:6400	3.5	3.1	113%
IgG	2	1:100	80.9		
		1:200	42.0	40.5	104%
		1:400	21.1	20.2	104%
		1:800	10.7	10.1	106%
		1:1600	5.6	5.1	110%
		1:3200	2.8	2.5	112%
IgM	3	1:100	97.6		
		1:200	49.0	48.8	100%
		1:400	23.2	24.4	95%
		1:800	13.4	12.2	119%
		1:1600	6.4	6.1	105%
		1:3200	3.0	3.1	97%
IgM	4	1:200	70.3		
		1:400	33.5	35.2	95%
		1:800	18.6	17.6	106%
		1:1600	10.1	8.8	115%
		1:3200	5.4	4.4	123%

Precision

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations each:

anti-β2-Glycoprotein I (IgG)		
Intra-Assay		
Sample No	Mean [U/ml]	CV [%]
1	13.4	5.0
2	24.3	2.1
3	88.0	2.8

anti-β2-Glycoprotein (IgM)		
Intra-Assay		
Sample No	Mean [U/ml]	CV [%]
1	14.7	3.8
2	30.0	2.5
3	67.9	2.1

Inter-Assay		
Sample No	Mean [U/ml]	CV [%]
1	11.0	7.4
2	29.9	7.9
3	94.9	2.6

Inter-Assay		
Sample No	Mean [U/ml]	CV [%]
1	15.7	6.3
2	32.6	1.1
3	82.9	4.3

Sensitivity

The lower detection limits for Anti-beta-2-Glycoprotein I IgG/IgM were determined at 0.5 U/ml.

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 endogenous 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, USA, IRP 97/656 and HCAL/EY2C9.These sera test positive for Anti-β2-Glycoprotein I autoantibodies.

LIMITATIONS OF PROCEDURE

The Anti-beta-2-Glycoprotein I IgG/IgM 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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Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Ογκος/αριθ..