DRG® Anti-ß2-Glycoprotein 1 IgA (EIA-3884)

Revised 23 May 2011 rm (Vers. 2.1) **RUO in the USA**

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

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

NAME AND INTENDED USE

Anti-beta-2-Glycoprotein I IgA is an indirect solid phase enzyme immunoassay (ELISA) for measurement of I 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.

PRINCIPLE OF THE TEST

Highly purified beta-2-glycoprotein I is bound to microwells. Antibodies against this antigen, 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 IgA immunologically detects the bound patient's 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 colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgA antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. Do not interchange kit components from different lots.
2. 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.
3. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
4. Avoid contact with the stop solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
5. 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.)
6. 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.
7. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
8. Do not pipette by mouth.

9. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
10. 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.
6 vials, 1.5 ml each	**Calibrators** with IgA class Anti-beta-2-glycoprotein I antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN_3 <0.1% (w/w)) IgA: 0; 6.3; 12.5; 25; 50 and 100 U/ml. Ready to use.
2 vials, 1.5 ml each	Anti-beta−2-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 package 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 IgA, 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.

DRG® Anti-ß2-Glycoprotein 1 IgA (EIA-3884)

Revised 23 May 2011 rm (Vers. 2.1) **RUO in the USA**

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes 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-hemolysed. 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.

Revised 23 May 2011 rm (Vers. 2.1) **RUO in the USA**

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 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. Pipette **100 µl** of calibrators, controls and prediluted patient samples in duplicate into the wells.

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

SA-SF: standards A to F
P1, P2..patient samples 1, 2 ...
C1: positive control
C1: 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 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 IgA ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

REFERENCES / Literature

1. Roubey, R. A. S. Review Article: Autoantibodies to phospholipid-binding plasma proteins: a new view of Lupus Anticoagulants and other "antiphospholipid" autoantibodies.
   Blood 1994; Vol 84, No 9: 2854 - 2867.
2. Schousboe, I. Beta-2-Glycoprotein I: a plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway.
   Blood 1985; Vol 66, No 5: 1086 - 1091.
3. Lee, N. S. et al. Beta-2-Glycoprotein I - Molecular properties of an unusual apolipoprotein, Apolipoprotein H.
   J. Biol. Chem. 1983; Vol 258, No 8: 4765 - 4770.
4. Kandiah, D. A. et al. Epitope mapping studies of antiphospholipid antibodies and beta-2GPI using synthetic peptides.
   Lupus 1995; Vol 4, Suppl 1: S7 - S11.
5. Matsuura, E. et al. Molecular studies on phospholipid-binding sites and cryptic epitopes appearing on b2-glycoprotein I structure recognized by anti-cardiolipin antibodies.
   Lupus 1995; Vol 4, Suppl 1: S13 - S17.
6. Koike, T. Anticardiolipin Antibodies and beta-2-Glycoprotein I.
   Clinical Immunology and Immunopathology 1994; Vol 72, No 2: 187 - 192.
7. Roubey, R. A. S. et al. "Anticardiolipin" autoantibodies recognise beta-2-Glycoprotein I in the absence of phospholipid.
   J. Immunol., 1995; Vol 154: 954 - 960.
8. Wang, M.-X. et al. Epitope specificity of monoclonal anti-beta-2-Glycoprotein I antibodies derived from patients with the antiphospholipid syndrome.
   J. Immunol., 1995; Vol 155: 1629 - 1636.
9. Arvieux, J. et al. IgG2 subclass restriction of anti-beta-2-Glycoprotein I antibodies in autoimmune patients.
   Clin. Exp. Immunol. 1994; Vol 95: 310 - 315.
10. Matsuda, J. et al. Prevalence of beta-2-glycoprotein I antibody in systemic lupus erythematosus patients with beta-2-glycoprotein I dependent antiphospholipid antibodies.
    Ann. Rheum. Dis. 1995; Vol 54: 73 - 75.
11. Martinuzzo, M. E. et al. Anti beta-2 glycoprotein I antibodies: detection and association with thrombosis.
    Brit. J. Haematol. 1995; Vol 89: 397 - 402.

12. Balestrieri, G. et al. Anti-beta2-glycoprotein I antibodies: a marker of antiphospholipid syndrome ? Lupus 1995; Vol 4: 122 - 130.
13. Miyakis S, Lockshin M. D., Atsumi T., Branch D. W., Brey R. L., Cervera R., Derksen R. H. W. M., de Groot P. G., Koike T., Meroni P. L., Reber G., Shoenfeld Y., Tincani A., Vlachoyiannopoulos P. G., Krilis S. A. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006; 4: 295-306.

Version 2011-04-13~rm