DRG® ThromboCombo IgG/IgM (EIA-3883)

Revised 20 Nov. 2010 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

The ThromoCombo IgG/IgM assay is an enzyme immunoassay (EIA) intended to screen for the presence of IgG and IgM class autoantibodies against beta-2-glycoprotein I, cardiolipin, phosphatidyl serine, phosphatidyl inositol and phosphatidic acid in human serum or plasma

PRINCIPLE OF THE TEST

Highly purified cardiolipin, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid and human beta-2-glycoprotein I are 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 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.

WARNINGS AND PRECAUTIONS

1. This kit is intended for Research Use Only. Not for use in diagnostic procedures.
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.

DRG

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CE

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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. / 12 profiles
Qty.1	divisible **microplate** consisting of 12 modules of 8 wells coated with: Row A: reference antigen Row B: beta-2-glycoprotein I (beta-2-glycoprotein I) Row C: cardiolipin (with •beta-2-glycoprotein I) Row D: phosphatidyl serine (with beta-2-glycoprotein I) Row E: phosphatidyl inositol (with beta-2-glycoprotein I) Row F: phosphatidic acid (with beta-2-glycoprotein I) Row G: mixed antigens: see coating row C-F (with beta-2-glycoprotein I) Row H: mixed antigens: see coating row C-F (without beta-2-glycoprotein I). Ready to use.
6 vials, 1.5 ml each	Combined antiphospholipid **calibrators** in a serum/buffer matrix (PBS, NaN_3 <0.1% (w/w)) containing **IgG:** 0; 6.3; 12.5; 25; 50; 100 U/ml and **IgM:** 0; 6.3; 12.5; 25; 50; 100 U/ml. 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 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 deionised 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.

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

Assay layout

Row A	The wells of row A are coated with a reference antigen and serve to perform the standard curve.
Row B	The wells are coated with beta-2-glycoprotein I.
Row C	The wells are coated with cardiolipin (plus beta-2-glycoprotein I).
Row D	The wells are coated with phosphatidyl serine (plus beta-2-glycoprotein I).
Row E	The wells are coated with phosphatidyl inositol (plus beta-2-glycoprotein I).
Row F	The wells are coated with phosphatidic acid (plus beta-2-glycoprotein I).
Row G	The wells are coated with a mixture of cardiolipin, phosphatidyl serine, phosphatidyl inositol and phosphatidic acid (plus beta-2-glycoprotein I).
Row H	The wells are coated with a mixture of cardiolipin, phosphatidyl serine, phosphatidyl inositol and phosphatidic acid (without beta-2-glycoprotein I).

Procedure

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted samples.
2. For the determination of one class of autoantibodies pipette **100 µl** of calibrators and **7 x 100 µl** prediluted samples into the wells. *For determination of both IgG and IgM autoantibodies calibrators and samples have to be pipetted in duplicate.*

	1	2	3	4	5	6	7	8	9	10	11	12
A - Reference antigen	SA	SB	SC	SD	SE	SF	SA	SB	SC	SD	SE	SF
B – beta-2-glycoprotein	P1	P2	P3	P4	P5	P6	P1	P2	P3	P4	P5	P6
C - cardiolipin & beta-2-glycoprotein	P1	P2	P3	P4	P5	P6	P1	P2	P3	P4	P5	P6
D – Phosphatidyl serin & beta-2-glycoprotein	P1	P2	P3	P4	P5	P6	P1	P2	P3	P4	P5	P6
E - Phosphatidyinositol & beta-2-glycoprotein	P1	P2	P3	P4	P5	P6	P1	P2	P3	P4	P5	P6
F - Phosphatidic Acid & beta-2-glycoprotein	P1	P2	P3	P4	P5	P6	P1	P »	P3	P4	P5	P6
G - Mixture row C/D/E/F & beta-2-glycoprotein	P1	P2	P3	P4	P5	P6	P1	P2	P3	P4	P5	P5
H - Mixture row C/D/E/F without beta-2-glycoprotein	P1	P2	P3	P4	P5	P6	P1	P2	P3	P4	P5	P6

SA, SB...SF Calibrators A to F P1,P2... Samples 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.

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