DRG® Anti-Phosphatidic Acid IgG / IgM (EIA-3594)

CE

Revised 30 Jan. 2013 rm (Vers. 3.1) **RUO in the USA**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

NAME AND INTENDED USE

Anti-Phosphatidic Acid IgG/IgM is an ELISA test system for the quantitative measurement of IgG and IgM class autoantibodies against phosphatidic acid in human serum or plasma.
This product is intended for professional research use only.

PRINCIPLE OF THE TEST

Highly purified phosphatidic acid is bound to microwells saturated with beta-2-glycoprotein I. Antibodies against the coated antigen, if present in diluted specimen sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound specimen 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 antibodies present in the original sample.

CONTENTS OF THE KIT

Symbols		Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: PPA
CALIBRATOR A	1x 1.5 mL	Calibrator A 0 GPL-U/mL / 0 MPL-U/mL, containing serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 mL	Calibrator B 6.3 GPL-U/mL / 6.3 MPL-U/mL, containing phosphatidic acid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 mL	Calibrator C 12.5 GPL-U/mL / 12.5 MPL-U/mL, containing phosphatidic acid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 mL	Calibrator D 25 GPL-U/mL / 25 MPL-U/mL, containing phosphatidic acid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 mL	Calibrator E 50 GPL-U/mL / 50 MPL-U/mL, containing phosphatidic acid antibodies in a serum/buffer matrix (PBS, BSA, NaN_3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 mL	Calibrator F 100 GPL-U/mL / 100 MPL-U/mL, containing phosphatidic acid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 mL	Control high, containing phosphatidic acid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.

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CONTROL –	1x 1.5 mL	Control low, containing phosphatidic acid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 mL	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE G	15 mL	Enzyme Conjugate; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative Proclin 0.05%, light red. Ready to use.
CONJUGATE M	15 mL	Enzyme Conjugate; containing anti-human IgM antibodies, HRP labelled; PBS, BSA, detergent, preservative Proclin 0.05%, light red. Ready to use.
TMB	15 mL	TMB Substrate; containing 3,3', 5,5'-Tetramethylbenzidin, colorless. Ready to use.
STOP	15 mL	Stop solution; contains acid. Ready to use.
WASH	20 mL	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50X conc.
	1	Instruction for Use
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µL
- Vortex mixer
- Pipettes for 10 µL, 100 µL and 1000 µL
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 mL and 100 mL
- Plastic container for storage of the wash solution

This ELISA is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

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

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STORAGE AND STABILITY

- Store test kit at 2 °C - 8 °C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Shelf life of the unopened test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2 °C - 8 °C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20 °C - 28 °C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional research use only.
- 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.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:
  Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- Exposure controls / personal protection:
  Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid:
  Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

WASH Wash Buffer

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 mL prior to use.

DILUENT Sample Buffer P

Prior to use dilute the contents (20 mL) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 mL.

Preparation of samples

Dilute specimen samples **1:100** before the assay:
Put 990 µL of prediluted sample buffer in a polystyrene tube and add 10 µL of sample. Mix well.
Note: Calibrators / Controls are ready to use and need not be diluted.

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TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and specimen samples.

1. Pipette **100 µL** of calibrators, controls and prediluted specimen samples into the wells.
2. Incubate for **30 minutes** at room temperature (20 °C - 28 °C).
3. Discard the contents of the microwells and **wash 3 times with 300 µL** of wash solution.
4. Dispense **100 µL** of enzyme conjugate into each well.
5. Incubate for **15 minutes** at room temperature.
6. Discard the contents of the microwells and **wash 3 times with 300 µL** of wash solution.
7. Dispense **100 µL** of TMB substrate solution into each well.
8. Incubate for **15 minutes** at room temperature
9. Add **100 µL** of stop solution to each well of the modules
10. Incubate for **5 minutes** at room temperature.
11. **Read** the optical density at 450 nm (reference 600-690nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1	A	P1								
B	B	P2	B	P2								
C	C	P3	C	P3								
D	D	P4	D	P4								
E	E	P5	E	P5								
F	F	P6	F	P6								
G	C+	P7	C+	P7								
H	C-	P8	C-	P8								
	IgG	IgG	IgM	IgM								

P1, ... specimen sample, A-F calibrators, C+, C- controls

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of specimen samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

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LIMITATIONS OF THE PROCEDURE

Each laboratory should establish its own ranges according to applicable laboratory guidelines.

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Rev. 01/16/13cc