



# DIAGNOSTIC AUTOMATION, INC.

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See external label



2°C-8°C



Σ=96 tests



Cat # 2560-6

# Anti-Phospholipid Screen IgG/IgM

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<b>Cat # Number</b>	<b>2560-6</b>
<b>Test</b>	<b>Antiphospholipid Screen IgG/ IgM ELISA</b>
<b>Method</b>	<b>ELISA: Enzyme Linked Immunosorbent Assay</b>
<b>Principle</b>	<b>ELISA - Indirect; Antigen Coated Plate</b>
<b>Detection Range</b>	<b>0-100u/ml IgG and IgM</b>
<b>Sample</b>	<b>10µl serum</b>
<b>Specificity</b>	<b>Not Observed</b>
<b>Sensitivity</b>	<b>0.5 U/ml</b>
<b>Total Time</b>	<b>~ 60min</b>
<b>Shelf Life</b>	<b>12 months</b>

*\* Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

## NAME AND INTENDED USE

The Diagnostic Automation, Inc. Anti-Phospholipid Screen IgG/IgM assay is a quantitative enzyme immunoassay (EIA) intended to screen for the presence of IgG and IgM class autoantibodies against Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic acid and  $\beta$ 2-Glycoprotein I in human serum or plasma 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

A mixture of highly purified Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and human  $\beta$ 2-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 or IgM immunologically detectst 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 450nm.

## WARNINGS AND PRECAUTIONS

1. This kit is intended for Reserach Use only. Not for use in diagnostic proedures.
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 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. Dispite the classification as non-hazardous, we strongly recommended 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 control and/or pooled sera. During handling of all kit reagents, control and serum samples observe the existing legal regulations.

## CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	divisible <b>microplate</b> consisting of 12 modules of 8 wells each, coated with a mixture of highly purified phospholipids: Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and saturated with human $\beta$ 2-Glycoprotein I. <i>Ready to use.</i>

6 vials, 1.5 ml each	Combined Anti-phospholipid <b>Standards</b> in a serum/buffer matrix (PBS, NaN <sub>3</sub> <0,1% (w/w)) containing IgG: 0; 6,3; 12,5; 25; 50 100 GPL U/ml and IgM: 0; 6.3; 12.5; 25; 50; 100 MPL U/ml. Ready to use.
2 vials, 1.5ml each	Anti-phospholipid <b>Controls</b> in a serum/buffer matrix (PBS, NaN <sub>3</sub> <0.1% (w/w)). Positive (1) and Negative (2), for the respective concentrations see the enclosed package insert. <i>Ready to use.</i>
1 vial, 20 ml	<b>Sample Buffer</b> (Tris, NaN <sub>3</sub> <0.1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	<b>Enzyme Conjugate</b> solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human <b>IgG</b> ; labelled with horseradish peroxidase. <i>Ready to use.</i>
1 vial, 15 ml	<b>Enzyme Conjugate</b> solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human <b>IgM</b> ; labelled with horseradish peroxidase. <i>Ready to use.</i>
1 vial, 15ml	<b>TMB Substrate Solution.</b> Ready to use.
1 vial, 15 ml	<b>Stop Solution</b> (1 M hydrochloric acid). <b>Ready to use.</b>
1 vial, 20 ml	<b>Wash Solution</b> (PBS, NaN <sub>3</sub> <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 for 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 contaminations 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. For the determination of one class of autoantibodies pipet 100 µl of Standards, Controls and prediluted patient samples into the wells.  
For determination of both IgG and IgM autoantibodies standards, controls and patient samples have to be pipetted in two attempts.

	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

- Incubate for 30 minutes at room temperature (20 - 28 °C).
- Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
- Dispense 100 µl of Enzyme Conjugate (Anti-h-IgG or Anti-h-IgM) into each well.
- Incubate for 15 minutes at room temperature.
- Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
- Dispense 100 µl of TMB substrate solution into each well.
- Incubate for 15 minutes at room temperature.
- Add 100 µl of Stop Solution to each well of the modules and incubate for 5 minutes at room temperature.
- 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 color is stable for at least 30 minutes. Read optical densities during this time.**

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