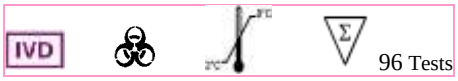
Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Anti-Phospholipid Screen IgG/IgM ELISA Kit

REF 2560-6



Test	Antiphospholipid Screen IgG/IgM ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Indirect; Antigen Coated Plate
Detection Range	0-100 µ/ml IgG and IgM
Sample	10 µL serum
Total Time	~ 60 min.
Shelf Life	12 Months from the manufacturing date
Specificity	Not Observed
Sensitivity	0.5 U/ml

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 ß2-Glycoprotein I in human serum or plasma as an aid in the diagnosis of an increased risk of thrombosis in patients with Systemic Lupus Erythematosis (SLE) or lupus-like disorders.

SUMMARY AND EXPLANATION

Screening for the presence of IgG and IgM class autoantibodies against Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic acid, and Beta2-Glycoprotein I in human serum or plasma as an aid in the diagnosis of an increased risk of thrombosis in patients with Systemic Lupus Erythematosis (SLE) or lupus-like disorders. SLE or lupus is a systemic autoimmune diseases that can affect any part of body. The immune system attacks the cells and tissue in the body causing inflammation and tissue damage. SLE mainly damage nervous system, heart, joints, skin, lungs, blood vessels, kidneys, and liver. Disease progress is however unpredictable with periods of illness alternating with remissions. The prevalence of disease is 9 times higher in females than males. Studies show that SLE may have genetic link and there is no cure for it.

TEST PRINCIPLE

A mixture of highly purified Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and human ß2-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 detects the bound patient antibodies forming a conjuagate/antibody/antigen complex. Washing of the microwells removes unbound conjuagate. 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.

SPECIMEN COLLECTION AND PREPARATION

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 activity.
6. Testing of heat-inactivated sera is not recommended.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate:** Package size 96 determ.Qty.1 divisible microplate consisting of 12 modules of 8 wells each, coated with a mixture of highly purified phospholipids: cardiolipin, phosphatidyl serine, phosphatidy lnositol,phosphatidic acid and saturated with human β2-glycoprotein I. Ready to use.
2. **Standards** 6 vials, 1.5 ml each Combined Anti-phospholipid Standard in a serum/buffer matrix (PBS, NaN3 <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.
3. **Control:** 2 vials, 1.5 ml each Anti-phospholipid Controls in a serum/buffer matrix (PBS, NaN3 <0.1% (w/w)).Positive (1) and Negative (2), for the respective concentrations see the enclosed QC insert. Ready to use.
4. **Sample Buffer**: 1 vial, 20 ml (Tris, NaN3 <0.1% (w/w)), yellow, concentrate (5x).
5. **Enzyme Conjugate IgG**: 1 vial, 15 ml (PBS, Proclin 300 <0,5% (v/v)), (light red) containing polyclonal anti-human IgG; labeled with horseradish peroxidase. Ready to use.
6. **Enzyme Conjugate IgM**: 1 vial, 15 ml (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal anti-human IgM; labeled with horseradish peroxidase. Ready to use.
7. **TMB Substrate Solution**: 1 vial, 15 ml Substrate Solution. Ready to use.
8. **Stop Solution**: 1 vial, 15 ml (1 M hydrochloric acid). Ready to use.
9. **Wash Solution**: 1 vial, 20 ml (PBS, NaN3 <0.1% (w/w)), concentrate (50x).

Materials required but not provided

1. Microplate reader capable for endpoint measurements at 450 nm
2. Multi-Channel Dispenser or repeatable pipet for 100 µl
3. Vortex mixer
4. Pipets for 10 µl, 100 µl and 1000 µl
5. Laboratory timing device
6. Data reduction software

REAGENT PREPARATION

1. Distilled or deionized water
2. Graduated cylinder for 100 and 1000 ml
3. Plastic container for storage of the wash solution

PROCEDURE NOTES:

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

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 regents 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 Standard 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 untill 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 assy.
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 pipette 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..			SA - SF:	standards A to F
D	SB	SF	P2	P..			P1, P2...	patient sample 1, 2 ...
E	SC	C1	P3				C1:	positive control
F	SC	C1	P3				C2:	negative control
G	SD	C2	P4					
H	SD	C2	P4					

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 color is stable for at least 30 minutes. Read optical densities during this time.

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-Phospholipid Screen 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 standard well. Use lin-log graph paper and plot the averaged optical density of each standard versus the concentration. Draw the best fitting curve approximating the path of all standard points. The standard points may also be connected with straight line segments. The concentration of unknown may then be estimated from the standard curve by interpolation.

Calculation Example

The figures below show typical results for Anti-Phospholipid Screen. These data are intended for illustration only and should not be used to calculate results from another run.

anti-PL	No	Position	OD 1	OD2	Mean	Conc.1	Conc. 2	Mean	decl.Con.	CV %
IgG	STA	A 1/B 1	0.051	0.049	0.050	0.3	0.1	0.2	0.0	3
IgG	STB	C 1/D 1	0.163	0.160	0.161	6.4	6.3	6.3	6.3	1
IgG	STC	E 1/F 1	0.310	0.273	0.291	12.8	11.2	12.0	12.5	9
IgG	STD	G 1/H 1	0.603	0.630	0.616	25	26	26	25	3
IgG	STE	A 2/B 2	1.122	1.054	1.088	51	47	49	50	4
IgG	STF	C 2/D 2	1.742	1.787	1.765	98	103	101	100	2
IgM	STA	A 7/B 7	0.022	0.021	0.022	0.2	0.1	0.2	0.0	3
IgM	STB	C 7/D 7	0.211	0.205	0.208	6.1	6.0	6.1	6.3	2
IgM	STC	E 7/F 7	0.465	0.462	0.464	13.0	12.9	13.0	12.5	0
IgM	STD	G 7/H 7	0.788	0.879	0.833	23	26	24	25	8
IgM	STE	A 8/B 8	1.411	1.382	1.397	52	50	51	50	1
IgM	STF	C 8/D 8	1.868	1.852	1.860	101	98	99	100	1

INTERPRETATION OF RESULTS

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the anti-Phospholipid Screen test:

Anti-Phospholipid-Ab

	IgG [GPL U/ml]	IgM [MPL U/ml]
normal:	< 10	< 10
elevated:	≥ 10	≥ 10

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 anti-Phospholipid antibodies.

PRECAUTIONS

1. This kit is intended for Reserach 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 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.

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ISO 13485
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Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

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