



AccuDiag™

Anti-Phospholipid Screen IgG/IgM

ELISA Kit

REF 2560

PIC AD2560ZT1

IVD See External Label 2°C 96 Tests

Anti-Phospholipid ELISA	
Principle	Indirect ELISA
Detection	IgG: 0-100 GPL-U/mL IgM: 0-100 MPL-U/mL
Sample	10 µL serum/plasma
Incubation Time	65 minutes
Sensitivity	IgG: 91.8% IgM: 53.4%
Specificity	IgG: 97.3% IgM: 96.7%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation, Inc. Antiphospholipid Screen IgG/IgM ELISA is a test system 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. This product is intended for professional in vitro diagnostic use only.

SIGNIFICANCE AND SUMMARY

Antiphospholipid syndrome (APS, Hughes Syndrome) is a systemic autoimmune disease that causes thromboses, recurrent miscarriage or stillbirths, and stroke.

Clinical symptoms are accompanied by specific autoantibodies in the blood, which bind to phospholipids like cardiolipin, or phospholipid-binding proteins like beta-2-glycoprotein I. Autoantibodies against proteins of the coagulation cascade, e.g., prothrombin or annexin V may also be found in patients with APS with otherwise negative phospholipid antibody results. In primary APS autoantibodies against phospholipids appear independently, while in secondary APS phospholipid antibodies are detected in conjunction with other autoimmune diseases, such as lupus erythematosus, rheumatoid arthritis, or Sjögren's syndrome.

ASSAY PRINCIPLE

A mixture of highly purified cardiolipin, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid and human beta-2- Glycoprotein I is bound to microwells.

The determination is based on an indirect enzyme linked immune reaction with the following steps:

Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components.

Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution, the bound enzyme conjugate hydrolyses the substrate forming a blue colored product. Addition of an acid stops the reaction generating a yellow end-product.

The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

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 or plasma by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2°C–8°C for up to five days or stored at -20°C for up to six months.
5. Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
6. Testing of heat-inactivated sera is not recommended.

MATERIALS AND COMPONENTS

Materials provided with the test kit

Sufficient for 96 determinations

MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use.
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 phospholipid 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 phospholipid 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 phospholipid 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 phospholipid 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 phospholipid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 mL	Control positive , containing phospholipid 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.
CONTROL -	1x 1.5 mL	Control negative , containing phospholipid 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 NaN ₃ 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'-Tetramethylbenzidine, colourless. Ready to use.
STOP	15 mL	Stop Solution ; contains acid. Ready to use.
WASH	20 mL	Wash Buffer , containing Tris, detergent, preservative NaN ₃ 0.09%; 50X conc.
	1	Instruction for Use
	1	Certificate of Analysis

Materials required but not provided

1. Microplate reader capable for endpoint measurements at 450 nm; optional: reference filter at 620 nm
2. Data reduction software
3. Multi-Channel dispenser or repeatable pipette for 100 µL
4. Vortex mixer
5. Pipettes for 10 µL, 100 µL and 1000 µL
6. Laboratory timing device
7. Distilled or deionised water
8. Measuring cylinder for 1000 mL and 100 mL
9. 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.

STORAGE AND STABILITY

1. Store test kit at 2°C - 8°C in the dark.
2. Do not expose reagents to heat, sun, or strong light during storage and usage.
3. Store microplate sealed and desiccated in the clip bag provided.
4. 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.
5. Diluted Wash Solution 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

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

REAGENT PREPARATION

Wash Buffer (WASH)

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

Sample Buffer P (DILUENT)

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

PREPARATION OF SAMPLES

Dilute all patient samples **1:100** with sample buffer prior to use in the assay. Put 990 µL of prediluted sample buffer in a polystyrene tube and add 10 µL of sample. Mix well.

Note: Calibrators and Controls are ready to use and need not be diluted.

ASSAY PROCEDURE

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

1. Pipette **100 µL** of calibrators, controls and prediluted patient 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.



- 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
- Incubate for **5 minutes** at room temperature.
- Read** the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed color 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	IgG	IgM								

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

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

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 patient 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.

PERFORMANCE CHARACTERISTICS

1. Calibration

Calibration is related to the internationally recognized reference sera from E.N. Harris, Louisville and to IRP 97/656 (IgG) and HCAL (IgG) / EY2C9 (IgM).

2. Measuring range

The calculation range of this ELISA assay is IgG: 0 - 100 GPL U/mL IgM: 0 - 100 MPL U/mL.

3. Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay:

Cut-off IgG: 10 GPL U/mL
IgM: 10 MPL U/mL

4. Interpretation of results

Negative: IgG < 10 GPL U/mL IgM < 10 MPL U/mL

Positive: ≥ 10 GPL U/mL ≥ 10 MPL U/mL

5. Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed [GPL-U/mL / MPL-U/mL]	Expected [GPL-U/mL / MPL-U/mL]	O/E [%]
IgG 1	1:100	98.0	98.4	100
	1:200	49.6	49.2	101
	1:400	24.3	24.6	99
	1:800	12.0	12.3	98
IgG 2	1:1600	5.8	6.2	94
	1:100	92.4	92.4	100
	1:200	45.9	46.2	99
	1:400	22.7	23.1	98
IgM 1	1:800	11.4	11.6	99
	1:1600	5.4	5.8	94
	1:100	92.7	92.7	100
	1:200	45.7	46.4	99
IgM 2	1:400	22.8	23.2	98
	1:800	11.2	11.6	97
	1:1600	5.4	5.8	93
	1:100	72.4	74.2	100
IgM 2	1:200	36.5	37.1	98
	1:400	18.7	18.6	101
	1:800	8.9	9.3	96
	1:1600	4.4	4.6	95

6. Limit of Detection

Functional sensitivity was determined to be:

IgG: 0.5 GPL-U/mL

IgM: 0.5 MPL-U/mL

7. Reproducibility

Intra-assay precision:

Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision:

Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay IgG		
Sample	Mean [GPL-U/mL]	CV [%]
1	10.4	5.1
2	18.7	3.4
3	59.9	5.2

Inter-Assay IgG		
Sample	Mean [GPL-U/mL]	CV [%]
1	10.0	3.6
2	17.7	5.4
3	57.9	4.9

Intra-Assay IgM		
Sample	Mean [MPL-U/mL]	CV [%]
1	12.8	4.1

Inter-Assay IgM		
Sample	Mean [MPL-U/mL]	CV [%]
1	12.6	5.3

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2	30.8	3.5
3	63.8	3.7

2	31.9	4.1
3	62.1	4.2

8. Interfering substances

No interference has been observed with hemolytic (up to 1000 mg/dL) or lipemic (up to 3 g/dL triglycerides) sera or plasma, or bilirubin (up to 40 mg/dL) containing sera or plasma.

Nor have any interfering effects observed with the use of anticoagulants (Citrate, EDTA, Heparin).

However, for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

9. Study results

Study Population	n	n Pos IgG	[%]	Pos IgM	[%]
Primary APS	8	7	87.5	6	75.0
Secondary APS	65	60	92.3	33	50.8
Normal human sera	150	4	2.7	5	3.3

Anti phospholipid IgG	Clinical Diagnosis		
		Pos	Neg
	Pos	67	4
Neg	6	146	
	73	150	223

Antiphospholipid IgM	Clinical Diagnosis		
		Pos	Neg
	Pos	39	5
Neg	34	145	
	73	150	223

Sensitivity: 91.8%
 Specificity: 97.3%
 Overall agreement: 95.5%

Sensitivity: 53.4%
 Specificity: 96.7%
 Overall agreement: 82.5%

LIMITATIONS OF THE ASSAY

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also, every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic 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, classification 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.



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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016	
	
ISO 13485 Quality Management for Medical Devices CERTIFIED	
 Diagnostic Automation/Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA	
Date Adopted	2025-05-22
Brand Name	AccuDiag™
REF 2560	AccuDiag™ - Antiphospholipid Screen IgG/IgM ELISA
PIC	AD2560ZT1
EU REP	AR Experts BV, Boeingavenue 209 1119 PD Schiphol-Rijk, The Netherlands info@ar-experts.eu
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