IMTEC-Phospholipid-Antibodies Screen

ELISA for the Quantitative Determination of Anti-Phospholipid Antibodies Ig(GM)

Package Size

REF	ITC59070	96 Tests	Complete Testkit
IVD			

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

DIL **DB01,** WASH 20x **WB06,** SUB **TMB ELISA and** STOP **STOP ELISA may be interchanged between lots and test kits that share the same reagent designation.**

All other reagents are specific for the individual test kit lot and must not be interchanged with other lots and test kits.

Store reagents at 2...8°C.

Intended Use

IMTEC-Phospholipid-Antibodies Screen is an enzyme immunoassay (ELISA) for the quantitative determination of IgG and IgM class autoantibodies against phospholipids in human serum. The assay is intended for professional in vitro diagnostic use only as an aid in the diagnosis of anti-phospholipid syndrome (APS).

Anti-Phospholipid-Antibodies are used for the diagnosis of the Anti-Phospholipid Syndrome (APS) and Systemic Lupus Erythematosus (SLE)[1,2], but may also be seen in other autoimmune disorders, like autoimmune hepatitis. The IMTEC-Phospholipid-Antibodies Screen ELISA detects the diagnostic relevant anti-phospholipid antibodies which have equal importance[3].

Testing for phospholipid antibodies is indicated in case of:

- systemic lupus erythematosus (SLE)
- suspicion of primary antiphospholipid syndrome (PAPS)
- suspicion of secondary antiphospholipid syndrome (SAPS)
- thrombophilia and spontaneous abortion in risk groups
- recurrent thrombophilia
- suspicion of thrombophilia or lupus-like diseases.

Principle

The test is based on the immobilisation of the phospholipids cardiolipin (CL), phosphatic acid (PA), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphathidylserine (PS) and human beta-2-glycoprotein I (b2GPI) to the solid phase of microtiter strips and subsequent binding of anti-phospholipid antibodies from patient serum.

The bound antibodies are detected with secondary antibodies that are directed against human IgG and IgM conjugated to horseradish peroxidase (HRP). After addition of substrate solution, a colour appears which intensity is proportional to the concentration of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow.

Reagents and Contents

MTP	12	**Microtiter Strips** (in 1 strip holder) 8-well snap-off strips, ready for use coated with CL, PA, PE, PI, PS and b2GP I
CAL	1 – 5 5 x 1.5 ml	**Calibrators Ig(GM)** (white cap), human serum, inked according to concentration, ready for use anti-phospholipid level: 6 U/ml **(1)**, 12 U/ml **(2)**, 25 U/ml **(3)**, 50 U/ml **(4)**, 100 U/ml **(5)**
NC	1.5 ml	**Negative Control Serum** (green cap), human, ready for use
PC	1.5 ml	**Positive Control Serum** (red cap), human, ready for use, coloured red Concentrations are stated on the labels.
WASH 20x WB06	50 ml	**Washing Buffer** (black cap) Concentrate (20x) for 1 l TRIS-buffer < 150 mM
DIL DB01	100 ml	**Dilution Buffer** (blue cap) ready for use Phosphate buffer < 10 mM

++++ Change of 📖 ++++ Please read marked text carefully! ++++

CON	15 ml	**Conjugate Solution** (white cap) anti-human IgG- and IgM-HRP-conjugate, ready for use, coloured red
SUB TMB ELISA	15 ml	**TMB solution** (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidine 1.2 mmol/l Hydrogen peroxide 3 mmol/l
STOP STOP ELISA	15 ml	**Stop Solution** (red cap) Sulphuric acid, ready for use 0.5 mol/l
	1	**Adhesive Strip, instructions for use, quick guide**

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to good laboratory practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days.

Reagent Preparation

Allow the testkit and all its components to reach room temperature before use!

Used bottles should be closed carefully and stored at 2...8°C. Store SUB protected from light.

Do not use polystyrene vessels for handling of CON.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

Washing Buffer Solution WASH

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part WASH 20x with 19 parts distilled water. WASH is stable for 6 weeks stored at 2...8°C.

Specimen

Patient sera

Use samples freshly collected or freeze samples at –20°C.

Allow the samples to reach room temperature (30 min).

Procedure

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add WASH and aspirate off again. Repeat washing twice.

W2: In case of automatic washers prime with WASH and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently (remaining liquid: < 15 µl).

W3: After washing, **remove remaining liquid by tapping** the plate upside down on tissue paper.

Pipetting scheme

Follow the procedure exactly as described.	
Reagents and specimens should be at room temperature before use.	
Sample Preparation: Dilute specimen 1:101 with reconstituted DIL (10 µl serum + 1 ml DIL); 100 µl is needed for each well.	
Step 1	***Volume per well [µl]***
CAL, PC, NC, sample	100
Seal MTP, incubate 1 hour at room temperature	
Discard solution	
Step 2	
Wash 3 times	
WASH	300
Step 3	
CON	100
Seal MTP, incubate 30 min. at room temperature	
Discard solution	

Step 4	
Wash 3 times	
WASH	300
Step 5	
SUB	100
Incubate 10 min. at room temperature	
Step 6	
STOP	100
Read absorbance values at 450 nm within the next 10 min. Bi-chromatic measurement with a reference wavelength at 620 – 690 nm is recommended.	

Automation

The IMTEC-Phospholipid-Antibodies Screen ELISA may be processed with suitable automated ELISA analyzers. Applications have to be validated prior to diagnostic use.

Validation of the Test

The test results are valid provided the following criteria are met for the obtained results:

- PC is within the indicated range (see label).
- NC is lower than the cut-off-value of the test.
- CAL 5 does not fall below an absorbance value of 1.0.
- The absorbances of CAL 1-5 keep rising.

In order to improve accuracy of the test results, we recommend to run CAL 1-5, PC, NC and patient samples in duplicate.

Interpretation of Results

Plot the measured absorbances against concentrations of CAL 1-5 in semi-log. By interpolating the plotted measuring points, a calibration curve is obtained, from which the concentrations of anti-phospholipid antibodies in the patient samples can be determined.

By use of computer assisted methods to construct the calibration curve, the 4-parameter curve fitting is recommended.

Results above 15 U/ml (cut-off value) for anti-phospholipid antibodies are considered positive, with concentrations below 25 U/ml having to be interpreted as borderline result.

Limitations

A positive result must be used in association with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for diagnosis only.

Elevated anti-phospholipid antibodies may occur in individuals with no evidence of clinical disease.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

According the international classification criteria a positive result should be confirmed with a new sample of the patient after 12 weeks[4].

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/el-59070.pdf or

www.human-de.com/data/gb/vr/el-59070.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Safety Notes

STOP **Warning**

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

SUB **Danger**

· Hazard statements

H360D May damage the unborn child.

· Precautionary statements

CAL NC PC WASH DIL CON SUB STOP

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Note

The handling should always be in compliance with common good laboratory practice requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

References

1. Cohen D. *et al.,* Diagnosis and management of the antiphospholipid syndrome, Brit Med J **340,** 1125-1132 (2010)
2. Favaloro E., Wong R. C. W., Laboratory testing for the anti-phospholipid syndrome: making sense of antiphospholipid antibody assays, Clin Chem Lab Med **49,** 447-461 (2011)
3. Galli M., Interpretation and recommended testing for anti-phospholipid antibodies, Semin Thromb Hemost **38**, 348-352 (2012)
4. Miyakis S. *et al.*, International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS), J Thromb Haemost **4** (2), 295-306 (2006)

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Human Gesellschaft für Biochemica und Diagnostica mbH
Max-Planck-Ring 21 · 65205 Wiesbaden · Germany
Telefon +49 6122-9988-0 · Telefax +49 6122-9988-100 · e-Mail human@human.de