

Test Instructions

IMTEC-Phosphatidylserine-Antibodies Combi

Enzyme Immunoassay for the Quantitative Determination of IgG and IgM Antibodies against Phosphatidylserine [HRP-conjugate: anti human (IgG resp. IgM)]

REF : TC 59022

Please read the instructions carefully before testing.

Procedural precautions:

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different lots.
- ▶ Store reagents at 2-8°C.

The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgG or IgM respectively. After addition of substrate solution, a color stain develops and its intensity is proportional to the concentration and/or the avidity of the detected antibodies.

1. Clinical Use

Over the past years, the determination of autoantibodies against phospholipids, detected as anti-cardiolipin antibodies or as lupus anticoagulant (LAC) has become increasingly important due to its association with arterial and venous thrombosis, tendency to abortion, neurological symptoms and signs as migraine and chorea, pulmonary hypertension as well as thrombocytopenia and hemolytic anemia. Following recently published clinical studies the determination of anti-phosphatidylserine antibodies is an essential criterion in differential diagnosis of patients with anti-cardiolipin antibodies. The determination of both antibodies should be carried out simultaneously. Anti-phosphatidylserine antibodies (aPs) are strongly associated with the Lupus anticoagulant. Nearly all patients with these multireactive autoantibodies, which are directed against both cardiolipin and phosphatidylserine, suffer from multiple thromboses in a sense of the anti-phospholipid syndrome.

2. Principle of the Test

The test is based on the immobilization of phosphatidylserine in a biological active vesicle like structure to a solid phase (polystyrene) and subsequent binding of the aPs. A better presentation of the antigenic epitop is achieved because of specially purified human β_2 -glycoprotein I (the anti-phospholipid cofactor) was added and the sample buffer also contains β_2 -glycoprotein I.

3. Materials Provided

-	MTP : phosphatidylserine coated microtiter strips (1x8), breakable	12 strips
-	CAL : standards aPs-IgG , ready to use,	500 μ L per vial
	1G 6.25 U/mL	1 vial each
	2G 12.5 U/mL	
	3G 25 U/mL	
	4G 50 U/mL	
	5G 100 U/mL	
-	CAL : standards aPs-IgM , ready to use,	500 μ L per vial
	1M 6.25 U/mL	1 vial each
	2M 12.5 U/mL	
	3M 25 U/mL	
	4M 50 U/mL	
	5M 100 U/mL	
	(all standards contain sodium azide and are inked according to concentration)	
-	CONTROL - : negative control serum, ready to use, contains sodium azide	1 vial 1 mL
-	CONTROL + G : positive control serum IgG , actual value see label, ready to use, contains sodium azide	1 vial 1 mL
-	CONTROL + M : positive control serum IgM , actual value see label, ready to use, contains sodium azide	1 vial 1 mL
-	BUF WASH 10x : washing buffer concentrate (10x)	1 bottle 50 mL
-	DIL SPE : sample buffer, ready to use	1 bottle 100 mL
-	CONJ a(hum IgG):HRP : Anti-human-IgG HRP conjugate, ready to use	1 bottle 6 mL

-	CONJ a(hum IgM):HRP :	1 bottle
	Anti-human-IgM HRP conjugate, ready to use	6 mL
-	SUBS TMB : TMB solution (HRP substrate), ready to use	1 bottle 12 mL
-	SOLN STOP : Stop solution, ready to use, sulfuric acid, handle with care, corrosive!	1 bottle 12 mL

4. Preparation of Reagents

Attention!

Allow the testkit and all its components to reach room temperature completely before executing it !

Please do not use any polystyrene vessels for handling of HRP conjugates.

If the test is performed automatically, we recommend the use of fresh conjugate each run and to discharge traces of old conjugate entirely. Remove washing buffer after washing steps completely.

4.1. Standards, Control Sera, Sample Buffer, HRP Conjugate, Stop Solution and TMB Solution

Standards, control sera, sample buffer, HRP-Conjugates, Stop solution and TMB solution are ready to use. Used bottles should be closed carefully and stored at 2-8°C. **Store TMB solution also protected from light.**

4.2. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part washing buffer concentrate **BUF** **WASH** **10x** with 9 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

4.3. Preparation of Serum or Plasma

Use serum or plasma freshly collected or freeze samples at -20°C. Do not use samples, that are repeatedly thawed and frozen. Do not use serum or plasma inactivated by heat treatment at 56°C. Allow the samples to reach room temperature (30 min). Dilute samples 1:100 with sample buffer **DIL** **SPE** (10 µL sample to 1 mL sample buffer **DIL** **SPE**).

4.4. Microtiter Strips

The strips are ready to use. Unused strips should be sealed in the lockable original bag at 2 - 8 °C.

5. Test Procedure

- **Pipette 100 µL serum or plasma dilution** or undiluted standards **CAL**, inked according to concentration, or control sera **CONTROL** **+** **G**, **CONTROL** **+** **M** and **CONTROL** **-**, into each well, for blanks use sample buffer **DIL** **SPE** instead of serum dilution, seal wells with adhesive foil.
- **Incubate for 1 hour** at room temperature (RT).
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.

- **Pipette 100 µL** HRP-Conjugate **CONJ** **a(hum IgG):HRP** or **CONJ** **a(hum IgM):HRP** into each well respectively, seal wells with adhesive foil.
- **Incubate for 30 minutes** at RT.
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µL TMB solution** **SUBS** **TMB** into each well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- **Pipette 100 µL stopping solution** **SOLN** **STOP** per well.
- **Measure at 450 nm** within the next 30 min after stopping.

6. Interpretation of Results

Plot the measured absorbances against concentrations of standards **CAL** (6.25 U/mL, 12.5 U/mL, 25 U/mL, 50 U/mL, 100 U/mL) in semi log. Determine the units of the examined samples from the standard curve directly. Results above 15 U/mL (cut-off value) for both IgG antibodies and IgM antibodies are considered positive. To prove the functionality of the test, the determined value for the positive control sera **CONTROL** **+** **G** and **CONTROL** **+** **M** are to be expected within the range labeled on the vial. The result of the negative control **CONTROL** **-** has to be lower than the cut-off value of the testkit.

Precautions

For in vitro diagnostics only.

IVD

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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