

IMTEC-CARDIOLIPIN-ANTIBODIES COMBI

ACL Combi

ELISA for the Quantitative Determination of Anti-Cardiolipin Antibodies (IgG and IgM)

Package Size

REF	ITC59082	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-Cardiolipin-Antibodies COMBI is an enzyme immunoassay (ELISA) for the quantitative measurement of IgG or IgM class autoantibodies against cardiolipin/beta-2-glycoprotein I in human serum and plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of antiphospholipid syndrome.

Anti-cardiolipin antibodies (aCL) are important for the diagnosis of the antiphospholipid syndrome (APS). In most patients with typical symptoms of APS these autoantibodies do occur. Therefore aCL are a diagnostic marker for the disease.

Testing for aCL is indicated in case of:

- 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 cardiolipin and purified human beta-2-glycoprotein I to the solid phase of microtiter strips and subsequent binding of anti-cardiolipin antibodies from patient serum.

The bound antibodies are detected with a peroxidase-labelled secondary antibody that is directed against human IgG or IgM. After addition of substrate solution, a colour appears which intensity is proportional to the concentration and/or the avidity of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow.

Contents

[MTP]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, ready for use coated with cardiolipin and purified human beta-2-glycoprotein I
[CAL] [G]	1 – 5 5 x 1.5 ml	Calibrators IgG (blue cap), human serum, coloured according to concentration, ready for use anti-cardiolipin level: 31.25 U/ml (1), 62.5 U/ml (2), 125 U/ml (3), 250 U/ml (4), 500 U/ml (5)
[CAL] [M]	1 – 5 5 x 1.5 ml	Calibrators IgM (white cap), human serum, coloured according to concentration, ready for use anti-cardiolipin level: 31.25 U/ml (1), 62.5 U/ml (2), 125 U/ml (3), 250 U/ml (4), 500 U/ml (5)
[NC]	1.5 ml	Negative Control Serum (green cap), human, ready for use
[PC] [G]	1.5 ml	Positive Control Serum IgG (red cap), human, ready for use Concentrations are stated on the labels.
[PC] [M]	1.5 ml	Positive Control Serum IgM (red cap), human, ready for use Concentrations are stated on the labels.
[WASH] 20x WB06	50 ml	Washing Buffer (black cap) Concentrate (20x) for 1 l

		TRIS buffer	pH 6.9 ± 0.2
[DIL] DB01	100 ml	Dilution Buffer (blue cap) ready for use Phosphate buffer	pH 7.3 ± 0.2
[CON] [G]	15 ml	Conjugate Solution (blue cap) anti-human-IgG HRP conjugate, ready for use	
[CON] [M]	15 ml	Conjugate Solution (red cap) anti-human-IgM HRP conjugate, ready for use	
[SUB] TMB ELISA	15 ml	TMB solution (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidin Hydrogen peroxide	pH 3.7 ± 0.2 1.2 mmol/l 3 mmol/l
[STOP] STOP ELISA	15 ml	Stop Solution (red cap) Sulphuric acid, ready for use	0.5 mol/l
	1	Adhesive Strip	

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.

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]** **[G]** and **[CON]** **[M]**.

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 or plasma

Use samples freshly collected or freeze samples at -20°C. **Freeze and thaw once only.** Do not use serum samples inactivated by heat treatment at 56°C.

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

Dilute samples 1:101 with **[DIL]** (add 10 µl sample to 1 ml **[DIL]**).

Procedure

- Pipette **100 µl** of **diluted patient sample**, **[CAL]**, **[PC]** and **[NC]**, into **[MTP]**, for blank use **[DIL]** instead of sample dilution, seal **[MTP]** with adhesive strip.
- Incubate for **1 hour** at RT.
- **Discard** the solution from **[MTP]**. **Wash** **[MTP]** 3 times using 300 µl **[WASH]** per well.
- **Discard** **[WASH]** and knock out residues on an absorbent paper or cloth.
- **Pipette 100 µl** **[CON]** **[G]** or **[CON]** **[M]** and seal **[MTP]** with adhesive strip.
- Incubate for **30 min.** at RT.
- **Discard** the solution from **[MTP]**. **Wash** **[MTP]** 3 times using 300 µl **[WASH]** per well.
- **Discard** **[WASH]** and knock out residues on an absorbent paper or cloth.
- **Pipette 100 µl** **[SUB]** and incubate for **10 min.** At room temperatures above 25°C the substrate incubation could be shortened, but should never fall short of 5 min..
- **Add 100 µl** **[STOP]** per well.
- **Read absorbance values at 450 nm** within the next 10 min. after stopping. Bi-chromatic measurement with a reference wavelength at 620 – 690 nm is recommended.

Automation

The IMTEC-Cardiolipin-Antibodies COMBI ELISA may be processed with suitable auto-mated 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|G** and **PC|M** are within the indicated range (see label).
- **NC** is lower than the cut-off-value of the test.
- **CAL|5G** and **CAL|5M** do not fall below an absorbance value of 0.6.
- The absorbances of **CAL|1G|5G** and **CAL|1M|5M** keep rising.

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

Interpretation of Results

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

Results above the respective cut-off values listed in the following table, are considered positive:

Unit	cut-off IgG	cut-off IgM
U/ml	48	44

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-Cardiolipin 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.

Performance Characteristics

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

www.human.de/data/gb/vr/el-59082.pdf or

www.human-de.com/data/gb/vr/el-59082.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|20x** **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.

References

1. Mackworth-Young C. G. *et al.*, Clin. Exp. Immunol. **136**, 393-401 (2004)
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3. Miyakis S. *et al.*, J. Thromb. Haemost. **4**, 295-306 (2006)
4. Luzzana C. *et al.*, J. Nephrol. **15**, 342-348 (2002)
5. Empson M. *et al.*, Cochrane Database Syst. Rev. **18**, CD002859 (2005)
6. Lassere M., Empson M., Thromb. Res. **114**, 419-426 (2004)
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EL-59082

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08-2018-16



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