# IMTEC-CARDIOLIPIN-ANTIBODIES IgM

## ACL IgM
## ELISA for the Quantitative Determination of Anti-Cardiolipin Antibodies (IgM)

**Package Size**

| | | | |
|---|---|---|---|
| REF | ITC59081 | 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, BUF WASH 10x WB06, SUBS TMB TMB ELISA and SOLN 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

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 to a solid phase (polystyrene) and subsequent binding of the aCL. A better presentation of the antigenic epitope is achieved because specially purified human $\beta_2$-glycoprotein I (the anti-phospholipid cofactor) was added and the sample buffer also contains $\beta_2$-glycoprotein I. The bound antibodies are detected with a peroxidase-labelled secondary antibody that is directed against human 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. The IMTEC calibrators are calibrated against the internationally accepted Sapporo standards (acc. to Koike et al., monoclonal antibody EY2C9).

### Reagents and Contents

| | |
|---|---|
| MTP | **12 Microtiter Strips** (in 1 strip holder)<br>8-well snap-off strips, coated with cardiolipin, ready for use |
| CAL 1 – CAL 5 | **5 x 1 ml Calibrators IgM** (white cap), human serum, inked according to concentration, ready for use<br>1: 31.25 U/ml<br>2: 62.5 U/ml<br>3: 125 U/ml<br>4: 250 U/ml<br>5: 500 U/ml |
| CONTROL - | **1 ml negative control serum** (green cap), human, ready for use |
| CONTROL + | **1 ml positive control serum** (red cap), human, ready for use<br>Concentrations are stated on the labels. |
| BUF WASH 10x<br>WB06 | **50 ml Washing Buffer** (black cap)<br>Concentrate (10x) for about 0.5 l<br>Phosphate buffer pH 6.7 ± 0.2 |
| DIL<br>DB01 | **100 ml Dilution Buffer** (blue cap)<br>ready for use,<br>Phosphate buffer pH 7.2 ± 0.2 |
| CONJ a(hum IgM):HRP | **12 ml Conjugate** solution (white cap) anti-human-IgM HRP conjugate, ready for use |
| SUBS TMB<br>TMB ELISA | **12 ml TMB Solution** (black cap)<br>ready for use, pH 3.7 ± 0.2<br>colourless to bluish<br>3,3',5,5'-tetramethylbenzidine 1.2 mmol/l<br>Hydrogen peroxide 3 mmol/l |
| SOLN STOP<br>STOP ELISA | **12 ml Stop Solution** (red cap)<br>Sulphuric acid, ready for use 0.5 mol/l |
| | **1 Pc 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.

SOLN STOP, SUBS TMB can irritate eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

### 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 SUBS TMB protected from light.

Do not use polystyrene vessels for handling of CONJ a(hum IgM):HRP.

**If the test is run on an automated system use fresh conjugate each time. Remove traces of old conjugate completely.**

### Washing Buffer Solution WASH

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part BUF WASH 10x with 9 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, CONTROL + and CONTROL - into MTP, for blank use DIL instead of sample dilution, seal MTP with adhesive strip.
- Incubate for **1 hour** at RT.
- **Wash** MTP using 250 µl WASH per well. **Repeat** procedure **3 times.**
- **Discard** WASH **and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µl** CONJ a(hum IgM):HRP and seal MTP with adhesive strip.
- Incubate for **30 min.** at RT.
- **Wash** MTP using 250 µl WASH per well. **Repeat** procedure **3 times**
- **Discard** WASH **and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µl** SUBS TMB 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** SOLN STOP per well.
- **Measure at 450 nm** within the next 30 min. after stopping.

### Validation of the Test

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

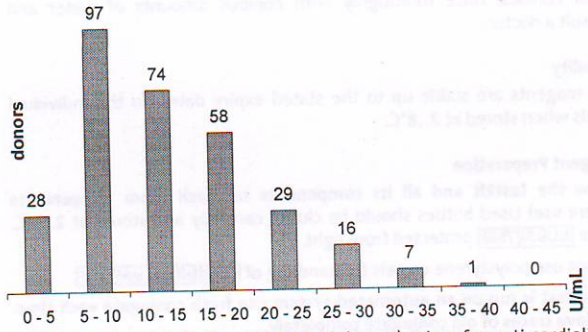
- CONTROL + is within the indicated range (see label).
- CONTROL - is lower than the cut-off-value of the test.
- CAL 5 does not fall below an absorbance value of 0.6.
- The absorbances of CAL 1 - 5 keep raising.

In order to improve accuracy of the test results we recommend to run CAL 1 - 5, CONTROL +, CONTROL - 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-cardiolipin antibodies in the patient samples can be determined. It is also possible to calibrate the test in ng/ml (IgM concentrations in CAL 1-5: 6.3 (**1**), 12.5 (**2**), 25 (**3**), 50 (**4**), 100 (**5**), ng/ml, acc. to Koike *et al.* – related to a sample dilution of 1:101) or MPL/ml (IgM concentrations in CAL 1-5: 7.1 (**1**), 14.2 (**2**), 28 (**3**), 57 (**4**), 114 (**5**), MPL/ml, acc. to Harris *et al.*) respectively. Using these, results above the respective cut-off values listed in the following table, are considered positive:

| Unit | cut-off IgM |
|---|---|
| U/ml | 44 |
| **ng/ml** | **8.8** |
| MPL/ml | 10 |




A corresponding distribution (310 blood donors) arises for anti-cardiolipin antibodies IgM.

**Limitations**

Sera from apparently normal blood donors may contain autoantibodies.

**Performance Characteristics**

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

www.human.de/data/gb/vr/el-59081.pdf or

www.human-de.com/data/gb/vr/el-59081.pdf

**Literature**


1. Conrad K. *et al.*, Autoantibodies in Systemic Autoimmune Diseases – A Diagnostic Reference; Pabst Science Publishers, Lengerich, Berlin, Riga, Rom, Viernheim, Wien, Zagreb, 2002
2. Bertolaccini M.L. *et al.*, Clin. Lab. **50**, 653-665 (2004)
3. Gromnica-Ihle E, Schößler W., Int. Arch. Allergy Immunol. **123**, 67-76 (2000)



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### Contents

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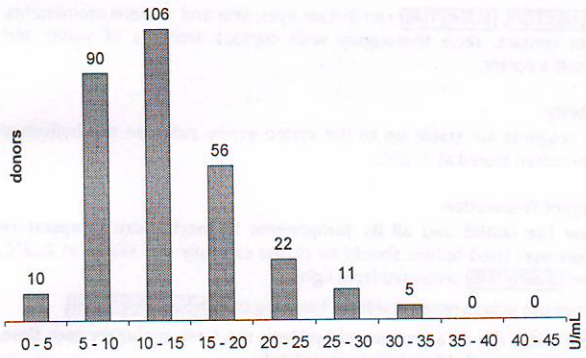
Plot the measured absorbances against concentrations of CAL 1-5 in semi-log. By interpolating the plotted measuring points, a calibration curve is obtained, from which the concentrations of the patient samples can be determined

It is also possible to calibrate the test in ng/ml (6.3 (**1**), 12.5 (**2**), 25 (**3**), 50 (**4**), 100 (**5**), ng/ml, acc. to Koike *et al.* – related to a sample dilution of 1:101) or GPL/ml (6.5 (**1**), 13 (**2**), 26 (**3**), 52 (**4**), 104 (**5**), acc. to Harris *et al.*) respectively. Using these, results above the respective cut-off values listed in the following table, are considered positive:

| Unit | cut-off IgG |
|---|---|
| U/ml | 48 |
| ng/ml | 9.6 |
| GPL/ml | 10 |




This histogram shows a determination of anti-cardiolipin antibodies IgG in 300 blood donors.

**Limitations**

Sera from apparently normal blood donors may contain autoantibodies.

**Performance Characteristics**

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

www.human.de/data/gb/vr/el-59071.pdf or

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