

Certificate

mdc medical device certification GmbH
certifies that

ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49 - 51
55129 Mainz
Germany

with the locations listed in the attachment

for the scope

**design, development, manufacturing and distribution of
in-vitro diagnostic test kits, reagents, controls and analyzers/instruments used
in the diagnosis of autoimmune and infectious diseases**

has introduced and applies a

Quality Management System

The mdc audit has proven that this quality management system
meets all requirements of the following standard

EN ISO 13485

Medical devices – Quality management systems –
Requirements for regulatory purposes

EN ISO 13485:2016 + AC:2016 - ISO 13485:2016

| | |
|------------------|------------------|
| Valid from | 2019-04-01 |
| Valid until | 2022-03-31 |
| Registration no. | D1227900020 |
| Report no. | P18-01487-133131 |
| Stuttgart | 2019-04-01 |



Head of Certification Body



Attachment of the certificate

No. D1227900020

date 2019-04-01

Page 1 of 1

| Location | Scope |
|--|--|
| ORGENTEC Diagnostika GmbH Carl-Zeiss-Straße 49 – 51 55129 Mainz Germany | design, development, manufacturing and distribution of in-vitro diagnostic test kits, reagents, controls and analyzers/instruments used in the diagnosis of autoimmune and infectious diseases |
| ORGENTEC Austria GmbH Hausfeldstraße 90 A2232 Deutsch-Wagram Austria | distribution of in-vitro diagnostic test kits, reagents, controls and analyzers/instruments used in the diagnosis of autoimmune and infectious diseases |
| ORGENTEC Hungary Kft. Aradi Vértanúk utca 45 H2060 Bicske Hungary | distribution of in-vitro diagnostic test kits, reagents, controls and analyzers/instruments used in the diagnosis of autoimmune and infectious diseases |



Head of Certification Body

EG Konformitätserklärung

EC Declaration of Conformity

ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49-51, 55129 Mainz, GERMANY

Wir erklären in eigener Verantwortung, dass das ORGENTEC Produkt
We declare in our sole responsibility that the ORGENTEC product

ORG 516 AMA-M2

zur quantitativen in-vitro-Bestimmung bestimmt ist und entsprechend Art. 9 Abs. Satz 1 der Europäischen Richtlinie 98/79/EG als „Sonstige Produkte“ (non-A, non-B, keine Selbstanwendung) klassifiziert ist.

as intended for use in quantitative in vitro determination is classified as "Other Devices" (non-A, non-B, no self-testing device) according to article 9 paragraph 1 sentence 1 of the European directive 98/79/EC.

Das Produkt stimmt mit den Grundlegenden Anforderungen und allen zutreffenden Bestimmungen der Richtlinie 98/79/EG des Europäischen Parlaments und des Rates vom 27. Oktober 1998 über in-vitro-Diagnostika überein. Die Konformität zur Richtlinie wurde durch ein Konformitätsbewertungsverfahren nach Anhang III der Richtlinie festgestellt.

This product is conform with the essential requirements and meet the appropriate provisions of the Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices. Conformity was proved by a conformity assessment procedure referred to in annex III of the directive.

Liste angewendeter Normen:

List of standards applied for CE marking:
EN ISO 13485, EN ISO 14971, EN ISO 18113, EN ISO 15223, EN ISO 23640, EN 13612.

Mainz, 2021-02-05

René Betz
Head of Regulatory Affairs




Gültig ab / Valid from 2021-02-05 bis / until 2024-02-28

Notification pursuant to §25 Abs. 3 Nr. 3 Medical Devices Act, MPG

Type: Reagent

EDMS 12-10-90-02-00

GMDN 43106

ORG 516_CE declaration of conformity_QM120330_2021-02-05_8

F4.01B Declaration of conformity

ORGENTEC Diagnostika GmbH

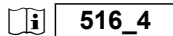
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ORG 516 AMA-M2

INTENDED PURPOSE

AMA-M2 is an ELISA test system for the quantitative measurement of IgG class autoantibodies against mitochondrial M2 subtype antigen in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

The test is used as an aid in the differential diagnosis of primary biliary cirrhosis (PBC). In patients with other autoimmune diseases occurrence of AMA antibodies may be related to the development or association of PBC. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS



In vitro diagnostic medical device



Manufacturer



Catalogue number



Sufficient for 96 determinations



Batch code



Use by



Temperature limitation



Keep away from sunlight



Do not reuse



Date of manufacture



CE marked according to 98/79/EC



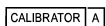
Consult instructions for use



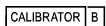
Electronic Instruction For Use: version



Microplate



Calibrator



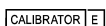
Calibrator



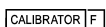
Calibrator



Calibrator



Calibrator



Calibrator



Control positive



Control negative



Sample Buffer P



Enzyme Conjugate



TMB Substrate



Stop solution



Wash Buffer



Ready to use

PRINCIPLE OF THE TEST

Highly purified mitochondrial M2 subtype (PDC-E2, BCOADC-E2, OGDC-E2) antigen 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 coloured 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.



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.

CONTENTS OF THE KIT

| | | | |
|--|---|-----------|---|
| ORG 516 |  | 96 | Sufficient for 96 determinations |
| MICROPLATE | | 1 | One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: AMA |
| CALIBRATOR A | | 1x 1.5 ml | Calibrator A 0 IU/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. |
| CALIBRATOR B | | 1x 1.5 ml | Calibrator B 12.5 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. |
| CALIBRATOR C | | 1x 1.5 ml | Calibrator C 25 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. |
| CALIBRATOR D | | 1x 1.5 ml | Calibrator D 50 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. |
| CALIBRATOR E | | 1x 1.5 ml | Calibrator E 100 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use. |
| CALIBRATOR F | | 1x 1.5 ml | Calibrator F 200 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. |
| CONTROL + | | 1x 1.5 ml | Control positive, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis. |
| CONTROL - | | 1x 1.5 ml | Control negative, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 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 sodium azide 0.09%, yellow, concentrate (5 x). |
| CONJUGATE | | 15 ml | Enzyme Conjugate containing anti-human IgG 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'- Tetramethylbenzidin, colorless. Ready to use. |
| STOP | | 15 ml | Stop solution; contains acid. Ready to use. |
| WASH | | 20 ml | Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc. |
|  | | 1 | Certificate of Analysis |

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay 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.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- 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.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C.
We recommend consumption on the same day.

PROCEDURAL NOTES

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

PREPARATION OF REAGENTS

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

DILUENT
Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

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

- Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
Incubate for **30 minutes** at room temperature (20-28 °C).
Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
- 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 600-690nm) and calculate the results.
The developed colour 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 | | | | | | | | | | |
| B | B | P2 | | | | | | | | | | |
| C | C | P3 | | | | | | | | | | |
| D | D | | | | | | | | | | | |
| E | E | | | | | | | | | | | |
| F | F | | | | | | | | | | | |
| G | C+ | | | | | | | | | | | |
| H | C- | | | | | | | | | | | |

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

Calibration

The assay system is calibrated against the international reference preparation WHO 67/183 for AMA-M2 as 100 IU/ml.

Measuring range

The calculation range of this ELISA assay is 0 - 200 IU/ml

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 10 IU/ml

Interpretation of results

Negative: < 10 IU/ml
Positive: ≥ 10 IU/ml

Linearity

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 IU/ml | Expected IU/ml | O/E [%] |
|--------|----------|----------------|----------------|---------|
| WHO | 1:100 | 108.5 | 100.0 | 109 |
| . | 1:200 | 51.2 | 50.0 | 102 |
| . | 1:400 | 25.2 | 25.0 | 101 |
| . | 1:800 | 12.8 | 12.5 | 102 |
| . | 1:1600 | 6.1 | 6.3 | 98 |
| . | 1:3200 | 3.1 | 3.1 | 99 |
| 1 | 1:100 | 49.5 | 49.5 | 100 |
| . | 1:200 | 25.0 | 24.8 | 101 |
| . | 1:400 | 12.2 | 12.4 | 99 |
| . | 1:800 | 5.9 | 6.2 | 95 |

Limit of detection

Functional sensitivity was determined to be: 1 IU/ml

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 | | |
|-------------|------------|------|
| Sample | Mean IU/ml | CV % |
| 1 | 39.8 | 7.0 |
| 2 | 81.3 | 3.8 |
| 3 | 177.3 | 3.6 |

| Inter-Assay | | |
|-------------|------------|------|
| Sample | Mean IU/ml | CV % |
| 1 | 40.1 | 6.2 |
| 2 | 84.6 | 11.8 |
| 3 | 180.4 | 3.8 |

Interfering substances

No interference has been observed with haemolytic (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 been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

| Study population | n | n Pos | % |
|---------------------------------|-----|-------|------|
| Primary biliary cirrhosis (PBC) | 143 | 139 | 97.2 |
| Rheumatoid Arthritis | 60 | 1 | 1.7 |
| Normal human sera | 267 | 18 | 6.7 |

| | | Clinical Diagnosis | |
|---------|-----|--------------------|-----|
| | | POS | NEG |
| ORG 516 | POS | 139 | 19 |
| | NEG | 4 | 308 |
| | | 143 | 327 |

Sensitivity: 97.2 %
Specificity: 94.2 %
Overall agreement: 95.1 %

LIMITATIONS OF THE PROCEDURE

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.

REFERENCES

1. Berg, P.A. and Klein, R. Diagnose der primär-biliären Zirrhose. IVD Nachrichten 1990; 1/1: 6 -7.
2. Berg, P.A. and Klein, R. Heterogeneity of anti-mitochondrial antibodies. Sem. Liver Dis. 1989; 9: 103 - 116.
3. Berg, P.A. and Klein, R. Immunology of primary biliary cirrhosis. Ballière's Clin.Gastroenterol. 1987; 1: 675 - 706.
4. Baum, H. and Palmer, C. The PBC specific antigen. Mol. Aspects Med. 1985; 8: 201 - 234.
5. Fussey, S.P.M., Guest, J.R., James, O.F W. et al. Identification and analysis of the major M2 autoantigens in primary biliary cirrhosis. PNAS, USA 1988; 85: 8654 - 8658.

Notice to the user (European Union):

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control

Former version: *ORG 516_IFU_EN_QM113145_2013-12-16_2.1* Reason for revision: *Introduction electronic IFU on homepage*

- 1 Pipet **100 µl** calibrator, control or patient sample
→ Incubate for **30 minutes** at room temperature
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 2 Pipet **100 µl** enzyme conjugate
→ Incubate for **15 minutes** at room temperature
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 3 Pipet **100 µl** substrate solution
→ Incubate for **15 minutes** at room temperature
- 4 Add **100 µl** stop solution
→ Leave untouched for **5 minutes**
→ Read at **450 nm**