




Mitochondrial (AMA)-M2-Ab ELISA

Enzyme immunoassay for the quantitative detection of antibodies
against M2 in human serum.

REF **RE70931**

 **96**

   **2-8 °C**

EU: **IVD**  U.S.: *For research use only.*
Not for use in diagnostic procedures.



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Instruction manual

Contents

1. Intended Use.....	2
2. Clinical Applications and Principle of the Assay.....	2
3. Kit Contents.....	3
4. Storage and Shelf Life.....	3
5. Precautions of Use.....	4
6. Sample Collection, Handling and Storage.....	4
7. Assay Procedure.....	5
8. Quantitative Interpretation.....	6
9. Technical Data.....	6
10. Performance Data.....	7
11. Literature.....	7
Pipetting scheme.....	8

1. Intended Use

Anti-M2-Ab ELISA is a solid phase enzyme immunoassay employing native mitochondrial M2 antigen for the quantitative detection of antibodies against M2 in human serum. The assay is a tool in the diagnosis of the primary biliary cirrhosis (PBC).

2. Clinical Application and Principle of the Assay

Primary biliary cirrhosis (PBC) is a chronic inflammatory disorder of the small and medium bile ducts and serologically characterized by the presence of circulating M2 autoantibodies. Anti-M2 autoantibodies belong to the group of anti-mitochondrial antibodies (AMA). The heterogeneously reacting specific AMA of the M2 subtype are directed against three related proteins of the α -keto acid dehydrogenase complex, which is located at the inner mitochondrial membrane. The major epitope recognized is located on the E2 subunit and the protein X of the pyruvate dehydrogenase complex (PDC). Additionally, AMA-M2 autoantibodies recognize the (E1 α and E1 β) subunits of the same complex and the E2 subunit of several other multienzyme complexes, such as the 2-oxo-glutarate dehydrogenase complex (OGDC) and the branched chain 2-oxo acid dehydrogenase complex (BCOADC).

The determination of AMA-M2 is a powerful tool in diagnosing PBC.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

3. Kit Contents

To be reconstituted:

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
Containing: Tris, NaCl, BSA, sodium azide (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide (preservative)

Ready to use:

Negative Control 1 vial, 1.5 ml (capped green: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Calibrators 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml
(color increasing with concentration: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Conjugate 1 vial, 15 ml IgG (capped blue: blue solution)
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H₂O₂

Stop Solution 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid

Microtiterplate 12x8 well strips with breakaway microwells
Coating see paragraph 1

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware, test tubes for dilutions. Distilled water. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette. Microplate washing device (multichannel pipette or automated system), adsorbent paper.

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C, at least. ***Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.***

5. Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics shall perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Reagents may be irritating to eyes and skin thus avoid contact with eyes and skin and wear disposable gloves. Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth. All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results. Allow all components to reach room temperature (20-26°C) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test. Always pipet substrate solution with clean tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in dry tubes. After separation, the serum samples should be used immediately, respectively stored at 2-8°C up to three days, or frozen at -20°C for longer periods.

7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

Washing

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells.

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C).

7.2 Work flow

- Pipette 100 µl of each patient's diluted serum into the designated microwells.
- Pipette 100 µl calibrators and negative and positive controls into the designated wells.
- Incubate for 30 minutes at room temperature (20-26°C).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 15 minutes at room temperature (20-26°C).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 15 minutes at room temperature (20-26°C), in the dark.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8. Quantitative Interpretation

Establish the standard curve by plotting the **optical density (O.D.) of each calibrator (y-axis)** with respect to the corresponding concentration values in **U/ml (x-axis)**. For best results we recommend log/lin coordinates and 4-Parameter Fit. From the O.D. of each sample, read the corresponding antibody concentrations expressed in **U/ml**.

Normal Range	Positive Results
< 15 U/ml	> 15 U/ml

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators IgG		O.D. 450/620 nm	CV %
0	U/ml	0.032	2.8
3	U/ml	0.152	2.6
10	U/ml	0.281	1.2
30	U/ml	0.646	2.4
100	U/ml	1.214	1.7
300	U/ml	2.104	1.6

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations. **Do not use this example for interpreting patients results!**

9. Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	60 minutes at room temperature (20-26°C)
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C use original vials, only
Number of determinations:	96 tests

10. Performance Data

10.1 Analytical sensitivity

The analytical sensitivity of this kit has been found at 1.0 U/ml.

10.2 Specificity

The microplate is coated with highly purified mitochondrial M2 antigen.

No crossreactivities to other autoantigens have been found.

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

10.4 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11. Literature

1. **Berg PA, Klein R (1989).**
Heterogeneity of antimitochondrial antibodies.
Seminars in liver disease 9: 103-116.
2. **Berg PA et al. (1982).**
ATPase associated antigen (M2): marker antigen for serological diagnosis of primary biliary cirrhosis.
Lancet II: 1423-1426.
3. **Manns M, Meyer zum Büschenfelde K-H (1989).**
Primäre biliäre Zirrhose.
In: Hepatologie und Praxis, Meyer zum Büschenfelde K-H, Arnold W, Hüttenroth TH eds.
Georg Thieme Verlag stuttgart, New York, 350-358.
4. **Coppel RL, Gershwin ME (1995).**
Primary biliary cirrhosis: The molecule and the mimic.
Immunological Reviews 44: 17-49.

Pipetting Scheme

	Calibrators (A-F)	Controls	Samples
Pipette Pipette Pipette	Calibrators (A-F) Controls Prediluted samples (1:101)	100 µl each 100 µl each	 100 µl each
Incubate	<i>30 min at room temperature (20-26°C)</i>		
Decant	<i>Wash 3x with 300 µl of wash buffer (1x)</i>		
Pipette	Conjugate	100 µl	100 µl
Incubate	<i>15 min at room temperature (20-26°C)</i>		
Decant	<i>Wash 3x with 300 µl of wash buffer (1x)</i>		
Pipette	Substrate	100 µl	100 µl
Incubate	<i>15 min at room temperature (20-26°C), in the dark.</i>		
Pipette	Stop Solution	100 µl	100 µl
Incubate	<i>5 min at room temperature (20-26°C)</i>		
<i>Agitate plate for 5 seconds and read OD at λ_{450} nm (optionally $\lambda_{450/620}$ nm) within 30 minutes. Resulting color is stable for 30 minutes, at least.</i>			

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

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LIABILITY: Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer