INSTRUCTION MANUAL

REF 4052

April 01, 2014

Anti-M2

- 96 determinations -

CE

IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG antibodies to the mitochondrial M2 antigen in human serum and plasma

REF	Catalogue number	**LOT**	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

Anti-M2 is used for the quantitative determination of IgG antibodies to the mitochondrial antigen M2 in human serum or plasma for the diagnosis of primary biliary cirrhosis (PBC).

The group of primary autoimmune liver disease (PAL) comprises autoimmune hepatitis (AIH), PBC and primary sclerosing cholangitis (PSC).

The clinical picture of PAL is in most cases not different from other chronic liver diseases. About 15% of all cases with chronic liver diseases show an autoimmune pathogenesis. Therefore, after exclusion of infectious etiology especially by viruses, the determination of different autoantibodies is recommended.

PBC is a chronic inflammatory disorder of the small and medium bile ducts and serologically characterized by the occurrence of circulating M2 autoantibodies. M2 autoantibodies belong to the group of mitochondrial antibodies (AMA) and recognize three related proteins of the alpha-keto acid dehydrogenase complex. This complex is located at the inner mitochondrial membrane. The major epitopes of this protein complex is found on the E2 subunit and the protein X of the pyruvate dehydrogenase complex (PDC). Furthermore, M2 autoantibodies bind the E1alpha and E1beta subunits of the same complex and the E2 subunit of several other multi-enzyme complexes, such as the 2-oxo-glutarate dehydrogenase complex (OGDC) and the branched chain 2-oxo acid dehydrogenase complex (BCOADC).

M2 autoantibodies have been found in up to 96% of patients suffering from PBC thus representing a powerful serological tool for the diagnosis of PBC.

Coppel RL, Gershwin ME: Primary biliary cirrhosis: The molecule and the mimic. Immunol Rev (1995) 44: 17-49

Berg PA, Klein R, Lindenborn-Fotinos J, Klöppel G: ATPase associated antigen (M2): marker antigen for serological diagnosisi of primary biliary cirrhosis. Lancet (1982) 2: 1423-1426

PRINCIPLE OF THE TEST

Anti-M2 is an enzyme immunoassay for the quantitative determination of IgG antibodies to the mitochondrial antigen M2.

The antibodies of the standards, controls, and diluted patient samples react with to the native mitochondrial antigen M2 immobilized on the solid phase of microtiter plates. The use of highly purified native mitochondrial antigen M2 guarantees the specific binding of M2 antibodies of the specimen under investigation. Following an incubation period of 30 min at room temperature (RT), unbound serum components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at RT. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethyl-benzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (HCl) into the wells after 30 min at RT turning the solution from blue to yellow.
The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at – 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.***

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells coated with native mitochondrial antigen M2 (bovine)	1 vacuum sealed with desiccant
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 50x	20 ml concentrate capped white
C DIL	**Concentrated sample diluent** 5x	20 ml concentrate capped white
D CONJ G	**Conjugate** containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped blue
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped black
F HCl	**Stop solution** 1.0 hydrochloric acid 1.0 M	15 ml ready for use capped white
1 - 6 CAL	**Standards** (diluted serum) conc.: 0, 3, 10, 30, 100, 300 U/ml	1.5 ml each ready for use
P CONTROL	**Positive control** (diluted serum) conc.: see leaflet enclosed +	1.5 ml ready for use capped red
N CONTROL	**Negative control** (diluted serum) conc.: see leaflet enclosed -	1.5 ml ready for use capped green

Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

Size and storage

Anti-M2 has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Anti-M2 have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

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Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 50 times with de-ionized or distilled water. For example, dilute 1 ml of the concentrate with 49 ml of distilled water per strip. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Prepare a sufficient amount of sample diluent by diluting the concentrated diluent 5 times with de-ionized or distilled water. For example, dilute 10 ml of the concentrate with 40 ml of distilled water. The sample diluent prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
   100 µl standards (1 - 6)
   100 µl positive (P) and negative (N) control
   100 µl diluted patient samples
   into the respective wells.
3. Incubate **30 min** at room temperature (18-25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) to each well.
6. Incubate **30 min** at room temperature (18-25°C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **30 min protected from light** at room temperature (18-25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

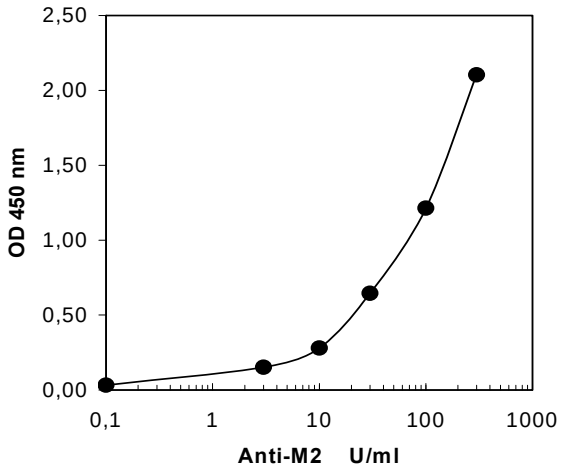
We recommend log / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the standards 1 - 6 on the ordinate, y-axis, (lin. scale) versus their respective Anti-M2 IgG concentrations on the abscissa, x-axis, (log. scale).
M2 antibody concentrations of the unknown samples are directly read off in U/ml against the respective OD values.
Anti-M2 may be used also with Computer Assisted Analysis using software able to plot log/lin curves with four-parameter fit.
Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Example of Typical Assay Results

Well	OD (a)	OD (b)	OD (mean)	U/ml
Standard **1**	0.031	0.033	0.032	**0**
Standard **2**	0.156	0.148	0.152	**3**
Standard **3**	0.275	0.287	0.281	**10**
Standard **4**	0.640	0.652	0.646	**30**
Standard **5**	1.226	1.202	1.214	**100**
Standard **6**	2.091	2.117	2.104	**300**
Patient **1**	0.794	0.792	0.793	**45**

TYPICAL STANDARD CURVE



Specimens with an OD > standard 6 should be diluted with M2 antibody negative serum and tested again. Results are multiplied with the dilution factor chosen.
Do not use this example for interpreting results.

Test validity

The test run is valid if:
- the mean OD of the standard 1 is ≤ 0.15
- the mean OD of the standard 6 is ≥ 1.3

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

M2 antibodies	U/ml
positive	**> 15**
negative	**≤ 15**

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum Anti-M2 levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the Anti-M2. However, M2 autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

No international reference material for this parameter is available thus the assay is calibrated in arbitrary units.

Linearity

Selected positive serum samples have been tested by this assay and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be sera that do not follow this rule.

Sensitivity

The analytical sensitivity of this assay was determined at 1.0 U/ml.

Specificity

No cross reactivity to other autoantigens have been found.

Precision

Intraassay variability

sample	mean IU/ml	standard deviation	CV (%)
1	153.3	5.2	3.4
2	63.7	1.8	2.8
3	24.8	0.34	1.4

Interassay variability

sample	mean IU/ml	standard deviation	CV (%)
1	144.8	6.1	4.2
2	59.7	1.2	2.1
3	21.4	0.32	1.5

INCUBATION SCHEME

Anti-M2 (4052)

Dilute patients sample **10 µl serum + 1.0 ml sample diluent (C)**

1	Bring all ready for use reagents to room temperature (18-25°C) before use.				
2	Pipette	Standards (1 - 6) Control (P + N) prediluted 1 + 100 patient sera	100 µl	100 µl	100 µl
3	Incubate		30 minutes at room temperature (18-25°C)		
4	Wash		Decant, 3 x 300 µl (made of B)		
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate		30 minutes at at room temperature (18-25°C)		
7	Wash		Decant, 3 x 300 µl (made of B)		
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light		30 minutes at room temperature (18-25°C)		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm within 30 min.				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some reagents contain small quantities of sodium azide (< 0.1%) as a preservative. Do not swallow reagents, and avoid contact with mucus membranes. Sodium azide can form explosive metal azides upon contact with lead and copper pipes, and therefore should therefore be disposed of with copious amounts of water.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.