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MESACUP-2 TEST Mitochondria M2

Cat. No. 7125E: 96 wells

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

5-10 Marunouchi 3 chome, Naka-ku, Nagoya, 460-0002 Japan

Tel: +81 52-971-2081 Fax : +81 52-971-2337 URL <http://www.mbl.co.jp>

English

INTENDED USE

The MESACUP-2 TEST Mitochondria M2 is semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti-Mitochondrial antibodies in human serum. The MESACUP-2 TEST Mitochondria M2 is intended for in vitro diagnostic use as an aid in the determination of certain autoimmune diseases.

SUMMARY AND EXPLANATION

Primary biliary cirrhosis (PBC) is an autoimmune liver disease named in 1950 by Ahrens et al. PBC is a disease caused by chronic inflammation of thin bile duct of the liver, making it difficult for the bile to flow, and the bile remains in the liver. Anti Mitochondrial antibodies occur frequently in patients with PBC and their presence constitutes one of the diagnostic criteria of the disease. Berg et al. classified corresponding antigen to AMA into M1-M9 and found anti M2 antibody is the most specific antibody to PBC. In 1988, it was reported by Gershwin, et al. that main corresponding antigen to anti M2 antibody is E2 component of pyruvate dehydrogenase complex (PDC). It is further reported that subunit (BCOADC-E2, OGDC-E2) of the enzyme which belongs to 2-acid dehydrogenase complex is also a corresponding antigen to M2 antibodies, and reported by Motegi et al. that antibodies which react only to one of them is present in approximately 5-6 % of sera from patient with PBC.

The MESACUP-2 Test Mitochondria M2 is for measuring anti- mitochondrial antibodies present in the serum with high sensitivity by ELISA.

PRINCIPLE

The MESACUP-2 TEST Mitochondria M2 measures anti-Mitochondrial antibodies present in the serum by ELISA. Calibrators, Controls and patient serum are added to microwell coated with mitochondrial M2 antigens, allowing anti-Mitochondrial antibody to react with the immobilized antigen (Sample incubation). After wash to remove any unbound serum proteins, horseradish peroxidase conjugated anti human IgG, IgA and IgM are added and incubated (Conjugate incubation). Following another washing step, the peroxidase substrate is added and incubated for an additional period of time (Substrate incubation). Acid solution is then added to each well to terminate the enzyme reaction and to stabilize the color development. The assay can be quantified by measuring the reaction photometrically and plotting the results.

BRIEF ASSAY PROCEDURE

<Sample incubation> Add 100 µl of diluted sample (1:101) to each well of microwell plate
(20-30°C) 60 min. ↓
Wash
↓
<Conjugate incubation> Add 100 µl of conjugate solution to each well
(20-30°C) 60 min. ↓
Wash
↓
<Substrate incubation> Add 100 µl of substrate to each well
(20-30°C) 30 min ↓
Add 100 µl of stop solution to each well
↓

Read absorbance

↓

Interpretation of result

REAGENTS AND STORAGE

1) MICROWELL STRIPS

96 wells MICROWELL STRIPS (8 x 12 wells) coated with antigens produced from recombinant purified proteins, the breakaway strips packaged in a strip holder and sealed in a foil envelope with desiccant, are stable at 2-8°C until the labeled expiration date.

2) CALIBRATOR 1 (0U/ml)

Two vials containing 1.5 ml of Assay Diluent including 0.09% sodium azide. Ready to use. Stable at 2-8°C until labeled expiration date.

3) CALIBRATOR 2 (100U/ml)

Two vials containing 1.5 ml each of anti-Mitochondrial antibody positive human serum with Assay Diluent including 0.09% sodium azide. Ready to use. Stable at 2-8°C until labeled expiration date.

4) CONJUGATE REAGENT

One vial containing 15 ml of horseradish peroxidase conjugated goat anti-human IgG, IgA and IgM (heavy chain specific), HEPES, Proclin 150 and BSA. Ready to use. Stable at 2-8°C until labeled expiration date.

5) ASSAY DILUENT

Two 50 ml bottles containing PBS, Tween-20, and 0.09% sodium azide. Ready to use. Stable at 2-8°C until labeled expiration date.

6) WASH CONCENTRATE (10X)

One 100 ml bottle containing PBS and Tween 20 as a 10X concentrate. Stable at 2-8°C until labeled expiration date.

7) SUBSTRATE SOLUTION

One 20 ml bottle containing 3,3',5,5'-tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H₂O₂). Ready to use. Stable at 2-8°C until labeled expiration date

8) STOP SOLUTION

One 20 ml bottle containing 1N Sulfuric acid. Ready to use. Stable at 2-8°C until labeled expiration date.

9) POSITIVE CONTROL SERUM

One vial containing 0.2 ml of anti-Mitochondrial antibody positive human serum with 0.09% sodium azide. Stable at 2-8°C until labeled expiration date.

10) NEGATIVE CONTROL SERUM

One vial containing 0.2 ml of anti-Mitochondrial antibody negative human serum with 0.09% sodium azide. Stable at 2-8°C until labeled expiration date.

PRECAUTIONS

- (1) This product is for in vitro diagnostic use only.
- (2) Do not use kit components beyond the stated expiration dates.
- (3) Avoid contact of reagents with eyes, skin and clothing. Reagents on skin must be washed away with plenty of water. TMB contains irritant and Stop Solution consists of a 1N sulfuric acid, which is a poison and corrosive.
- (4) Calibrator 2, Positive control and Negative control are derived from human serum, in which HBs antigen, HCV antibody and HIV antibody has not been detected. No test method, however, can guarantee the absence of these or any other infectious agents. These reagents and all patient samples should be handled as if they are capable of transmitting AIDS, hepatitis or any other infectious diseases.
- (5) Calibrator 1, Calibrator 2, Positive control, Negative control and Assay Diluent contain sodium azide (0.09%) as a preservative and must be handled with caution - do not ingest or allow contact with skin or mucous membranes. Sodium azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush with plenty of water when disposing materials containing sodium azide into a drain.
- (6) Some kit components contain animal origin materials, which are from non-infectious animals. These components, however, should be treated as potential biohazards in use and for disposal.
- (7) Matching lot numbers of Microwell strips, Conjugate and Calibrator 2 must be used together in the assay. Do not substitute reagents from other kits.
- (8) All reagents must be brought to room temperature (20-30°C) before starting the assay.
- (9) Do not expose the kit to direct sun during assay and storage.
- (10) Avoid microbial and cross contamination of reagents or samples.
- (11) Incubation temperatures above or below normal room temperature (20-30°C), shorter or longer time periods of incubation and inaccurate dilution may give erroneous results.
- (12) The wells must be rinsed with Wash Solution properly enough to avoid false positive.
- (13) Carefully pipette not to foam each sample and reagent to avoid cross contamination between microwells.
- (14) All microwell strips, which are not immediately required, should be returned to the zip lock pouch, which must be carefully resealed to avoid moisture absorption.
- (15) Wash concentrate may become turbid at 2-8°C, which does not cause inconsistent results.
- (16) Implement used for the test should be disposed or treated as shown below.

Soak in 2% final conc. glutaraldehyde solution for more than 1 hour or soak in 0.5% Sodium hypochlorite solution (available chlorine: approx. 5000ppm) for more than 1 hour or autoclave at 121°C for more than 20 minutes.
- (17) The Mitochondrial antibodies value obtained from this assay are an aid to diagnosis only. Each physician must interpret these results in light of the patient's history, physical findings, and other diagnostic procedure.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader (wavelength: 450 nm, 620 nm/reference)
- Multichannel micropipette (e.g. 100 µl - 300 µl) for dispensing conjugate, substrate, and stop solution.
- Single channel pipette (10 µl & 100 µl)
- Reagent reservoir
- Autowasher or wash bottle

- Deionized or distilled water
- One liter graduated cylinder for preparation of wash solution
- Test tubes for patient sample dilutions (e.g. 1000 µl)
- Disposable pipette tips
- Paper towels
- Basin and disinfectant
- Microplate cover

PROCEDURE

■ PREPARATION OF REAGENTS

- Bring all assay materials to room temperature (20-30°C) prior to use.
- Microwell Strips: Remove required microwell strip from pouch and place them in the frame. Promptly return unused strips to refrigerated storage.
- Wash Solution: Prepare 1:10 dilution of The Wash Concentrate prior to use.(ex. add 100 ml of Wash Concentrate to 900 ml of distilled water). The diluted wash solution is stable for 2 weeks at 2 -8°C.
- Do not dilute Calibrator 1, Calibrator 2, Conjugated Reagent, Assay Diluent, Substrate and Stop Solution, which are ready-to-use.

■ PREPARATION OF SAMPLES/CONTROLS

- Dilute each patient serum, Positive Control and Negative Control 1:101 by adding 10µl of serum to 1ml of Assay Diluent.
 - *Diluted samples must be used within a day.
 - * Assay Diluent may form precipitate, which does not cause inconsistent results.
- The controls should be treated in the same way as a patient serum.
- Use fresh patient sera. If storage is needed, they should be frozen below -20°C for up to one month, below -70°C for longer storage. Do not repeat freezing and thawing.
 - *In case stored below -20°C for more than 6 months or freezing and thawing repeatedly, nonspecific results are obtained because of IgG denaturation.

■ ASSAY PROCEDURE

STEP 1. (SAMPLE INCUBATION)

Using the multi-channel pipetor, transfer 100µl of each diluted sample, Positive and Negative Controls into the appropriate microwells of the antigen test plate. Add Calibrators directly to appropriate wells. (Do not dilute Calibrators.)

- * Incubation starts on pipetting to the antigen-coated microwells. Pipetting should be completed as quickly as possible.

Cover wells with a plate sealer and incubate for 60 minutes at room temperature (20-30°C).

STEP 2. (WASHING)

Aspirate or discard the well contents. Fill the well with Wash Solution and then completely aspirate or discard the contents. Wash 4 times. Tap the plate on a paper towel to remove any remaining Wash Solution. When autowasher is used, wash 4 times.

- * Each laboratory is recommended to confirm its own appropriate washing times and set- up.
- * Wash Solution should be used at 20-30 °C.

STEP 3. (CONJUGATE INCUBATION)

Pour Conjugated Reagent into a reservoir. Add 100 µl of the Conjugated Reagent to each well with

multichannel pipette. Cover wells with the plate sealer and incubate for 60 minutes at room temperature (20-30°C).

STEP 4. (WASHING)

Wash the microplate following the STEP 2 procedure.

STEP 5. (SUBSTRATE INCUBATION)

Pour Substrate into a reservoir and pipette 100 µl of the Substrate to each well with multichannel pipette.

- * The reservoir should be different from the one, which was used for pouring conjugate solution. A new disposable reservoir should be used because Substrate is easily oxidized by metal ion.
- * The Substrate, once poured in a reservoir, should not be returned to the bottle.

Cover wells with the plate sealer and incubate for 30 minutes at room temperature (20-30°C).

STEP 6. (STOP REACTION)

Pour Stop Solution into a reservoir. Pipette 100 µl of the solution to each well with multichannel pipette.

■ READING

Read the absorbance of each well at 450 nm. If a dual wave length platereader is available, set the test wavelength at 450 nm and the reference at 620 nm.

- * Reading should be done as quickly as possible after stopping the reaction.
- * Ensure that the bottom of the plate is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before reading the plate.

■ CALCULATION OF RESULT

$$\text{Unit value (U/ml)} = \frac{(A_{450}\langle\text{Sample}\rangle - A_{450}\langle\text{Calibrator 1}\rangle)}{(A_{450}\langle\text{Calibrator 2}\rangle - A_{450}\langle\text{Calibrator 1}\rangle)} \times 100$$

*A₄₅₀ is abbreviation of absorbance value at 450 nm.

*An international reference material for anti-mitochondria antibodies is not available. The assay is calibrated in relative arbitrary units.

■ QUALITY CONTROL

Each assay result should meet the following criteria.

A₄₅₀ of Calibrator 1: ≤0.15

A₄₅₀ of Calibrator 2: ≥0.70

The Positive and Negative Controls must give the following results:

	Positive Control	Negative Control
Anti-Mitochondrial Ab Value (U/ml)	> 50	< 7

If any of these are not met, the results are invalid and the test should be repeated.

Before repeating assay, check the following procedure.

- Incubation Temperature
- Incubation Period of Time
- Washing

TEST INTERPRETATION AND EXPECTED VALUES

The following is intended only as a guide for interpretation. Each laboratory is recommended to establish its own criteria for test interpretation based on sample populations typically encountered.

Anti-mitochondrial Ab value (U/ml)	Interpretation
< 7	Negative for anti-mitochondrial Ab
Greater than or equal to 7	Positive for anti-mitochondrial Ab

■ LIMITATIONS

As with other diagnostic test procedures, the results obtained with the MESACUP-2 TEST Mitochondria M2 serve only as an aid to diagnosis and should not be interpreted as diagnostics in themselves.

PERFORMANCE CHARACTERISTICS

■ CLINICAL SPECIFICITY AND SENSITIVITY

Disease	Positive sample	Positive rate
PBC	111/123	90.2%
AIH I	1/27	3.7%
AIH II	0/4	0.0%
HBV	0/44	0.0%
HCV	2/46	4.3%
Normal Serum	3/168	1.8%

PBC: Primary biliary cirrhosis

AIH: Autoimmune hepatitis

HBV: Hepatitis B virus

HCV: Hepatitis C virus

■ PRECISION

Repeatability was demonstrated by testing 3 samples in sextuple. Reproducibility was determined by testing 3 samples on 5 different days (day-to-day) and by testing 3 samples in 3 lots (lot-to-lot). %CV values for reproducibility and repeatability were below 15% for each sample.

■ ASSAY RANGE

The assay range of this kit is from 5U/ml to 300U/ml.

■ INTERFERING SUBSTANCES

Hemoglobin (up to 480 mg/dl), Bilirubin (up to 20.0mg/dl), chyle (up to 2,780 unit as Formazine) and/or Rheumatoid factor (up to 520 IU/ml) are not affective on the assay result, but avoid using highly hemolysed samples or highly lipemic samples.

REFERENCES

1. Berg, P.A. et al : Antimitochondrial antibodies in primary biliary cirrhosis. J.Hepatol, 1986, 2 :123
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3. Leung PS, et al : Autoantibodies to BCOADC-E2 in patients with primary biliary cirrhosis recognize a conformational epitope. Hepatology 1995, 22: 505-513
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Manufactured by:

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

5-10 Marunouchi 3-chome, Naka-ku, Nagoya 460-0002, JAPAN

Tel: +81 52-971-2081

Fax: +81 52-971-2337

Authorized Representative in the EU:

QARAD b.v.b.a.

Volmolenheide 13, 2400 Mol, Belgium

	<u>www.e-labeling.eu/MBL009279</u>
	+800 135 79 135 GR 00 800 161 220 577 99

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