

Test Instructions IMTEC-AMA M2

Enzyme Immunoassay for the Quantitative Determination of Anti-Mitochondrial M2 Antibodies

REF : TC 60040

Please read the instructions carefully before testing.

Procedural precautions:

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different lots.
- ▶ Store reagents at 2-8°C.

1. Clinical Use

Antimitochondrial antibodies (AMA) are a heterogeneous group of antibodies which are directed against proteins of the outer and inner membrane of the mitochondria. The autoantigens, at which the AMAs are aiming, are present in different tissues (liver, kidney, stomach, heart) at varying concentrations. So far 9 different AMA-subtypes have been distinguished but their exact specificity has not yet been discovered in all cases.

Antimitochondrial M2-antibodies which are considered to be very specific for primary biliary cirrhosis (PBC) are aiming at the α -ketoacid-dehydrogenase-complex but the dominating epitop is localized on the pyruvate-dehydrogenase-complex (PDH).

Antimitochondrial M2-antibodies (AMA-M2) can be detected in about 90% of patients with primary biliary cirrhosis and they have due to that a very high specificity and sensitivity for this disease.

2. Principle of the Test

The test is based on the binding of the pyruvate dehydrogenase complex to the solid phase of microtiter strips and subsequent binding of AMA from patient serum. The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgG.

After the addition of substrate solution, a color develops. Its intensity is proportional to the concentration and/or the avidity of the detected autoantibodies.

3. Materials Provided

-	MTP	: PDH-coated microtiter strips (1 x 8), breakable, ready to use	12 strips + frame
-	CAL	: standards, ready to use	1 vial each 750 μ L per vial
	1	2 U/mL	
	2	8 U/mL	
	3	31 U/mL	
	4	125 U/mL	
	5	500 U/mL	
		all standards contain sodium azide and are inked according to concentration	
-	CONTROL -	: negative control serum, ready to use, contains sodium azide	1 vial 1 mL
-	CONTROL +	: positive control serum, ready to use, contains sodium azide	1 vial 1 mL
-	BUF WASH 10x	: washing buffer concentrate (10x)	1 bottle 50 mL
-	DIL SPE 5x	: sample buffer concentrate (5x)	1 bottle 22 mL
-	CONJ a(hum IgG):HRP	: HRP-Conjugate, anti-human IgG, ready to use	1 bottle 12 mL
-	SUBS TMB	: TMB solution, HRP substrate, ready to use	1 bottle 12 mL
-	SOLN STOP	: stopping solution, ready to use, contains sulfuric acid, caution corrosive	1 bottle 12 mL

4. Preparation of Reagents

Attention!

Allow the testkit and all its components to reach room temperature completely before executing it !

Please do not use any polystyrene vessels for handling of HRP conjugates.

In case of running the testkit automatically, it is recommended to use fresh conjugate each time. Please remove traces of old conjugate completely.

4.1. Standards, control sera, HRP Conjugate, Stopping Solution and TMB Solution

Standards, control sera, HRP Conjugate, stopping solution and TMB solution are ready to use. Used bottles should be closed carefully and stored at 2-8°C. **Store TMB solution also protected from light.**

4.2. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part washing buffer concentrate [BUF] [WASH] [10x] with 9 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2 – 8 °C.

4.3. Preparation of Sample Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part sample buffer concentrate [DIL] [SPE] [5x] with 4 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2-8°C.

4.4. Preparation of Sera

Use freshly collected serum samples or freeze sera at -20°C until analysis. Allow the samples to reach room temperature (30 min). Dilute samples 1:100 with sample buffer (10 µL sample to 1 mL buffer).

4.5. Microtiter Strips

The strips are ready to use. Unused strips should be stored in the lockable original bag at 2-8°C.

5. Test Procedure

- **Pipette 100 µL serum dilution** or standards [CAL], inked according to rising concentration, or control sera [CONTROL] [+] and [CONTROL] [-] into each well, for blank use sample buffer instead of serum dilution, seal wells with adhesive foil.
- **Incubate for 1 hour** at room temperature (RT).
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Pipette 100 µL of HRP-conjugate** [CONJ] [a(hum IgG):HRP] into each well, seal wells with adhesive foil.
- **Incubate for 30 minutes** at RT
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Pipette 100 µL TMB solution** [SUBS] [TMB] into each well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- **Pipette 100 µL stopping solution** [SOLN] [STOP] per well.
- **Measure at 450 nm** within the next 30 min after stopping.

6. Interpretation of Results

Plot measured absorbances against concentrations/units of standards [CAL] (2 U/mL, 8 U/mL, 31 U/mL, 125 U/mL, 500 U/mL) in semi log. Determine the units of the examined samples from the standard curve directly.

Results in the range of 5-10 U/mL are borderline or slightly positive. Results above 10 U/mL (cut off) are considered positive.

To prove the functionality of the test, the value determined for the positive control serum [CONTROL] [+] is to be expected within the range labeled on the vial. The result of the negative control [CONTROL] [-] has to be lower than the cut off value of the testkit.

Precautions

For in vitro diagnostics only.

[IVD]

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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IMTEC Immundiagnostika GmbH

Robert-Rössle-Straße 10

13125 Berlin

GERMANY

Tel.: +49 (30) 94 89 36 00

Fax: +49 (30) 94 89 36 15

www.imtec-immundiagnostika.de

imtec@mdc-berlin.de