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User's Manual

AMA-M2 Ab ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

AMA-M2 Ab ELISA Kit is an indirect solid phase ELISA for the quantitative measurement of IgG class autoantibodies against mitochondrial M2 subtype antigen in human serum or plasma.

General Description

Anti-mitochondrial antibodies (AMA) are a heterogeneous group of autoantibodies directed against various proteins that are located in the outer and inner membrane of mitochondria. Specific anti-mitochondrial antibodies have been described for the primary biliary cirrhosis (PBC) as subtypes M2, M4, M8 and M9. Other AMA subtypes are related to other diseases, like collagenosis (AMA-M5) and drug induced LE and Hepatitis (AMA-M3 and AMA-M6).

The heterogeneously reacting specific anti-mitochondrial antibodies of the M2 subtype are directed against three related proteins of the α-keto acid dehydrogenase complex which is located at the inside of the mitochondrial membrane. The recognized major epitope is located on the E2 subunit and the protein X of the pyruvate dehydrogenase complex (PDC). Additionally AMA-M2 autoantibodies recognise the (E1a und E1b) 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).

Using HEp 2 Cell monolayers for indirect immune fluorescence AMA-M2 autoantibodies are characterised as a fine-speckled cytoplasmatic, perinuclear condensed fluorescence pattern. For differential diagnosis of the primary biliary cirrhosis (PBC) determination of AMA-M2 by ELISA is recommended because of its high sensitivity and specificity.

In patients with other autoimmune diseases determination of AMA antibodies allows an early screening for the occurence of subtype M2 and M9 antibodies which may be related with the development and / or association of PBC.

Profiling the AMA subtypes allows an immunological and prognostic classification of the primary biliary cirrhosis. Beginning cases of symptomatic PBC often exhibit only AMA-M2 sub-type antibodies (sometimes in combination with AMA-M9), whereas progressive cases and mixed syndromes with chronic acute hepatitis (CAH) are related with the occurrence of AMA-M2, -M4 and -M8 antibody subtypes.

Principles of Testing

Highly purified mitochondrial M2 subtype (PDC-E2, BCOADC-E2, OGDC-E2) antigen is bound to microwells. Antibodies against the coated antigen, if present in diluted serum, bind in the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detect the bound antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

Reagents And Materials Provided

1. Divisible microplate consisting of 12 modules of 8 wells each. Ready to use. -- 96 wells
2. Calibrators (A-F) (0,12.5, 25, 50,100, 200 IU/mL), containing serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. -- 1.5 mL × 6
3. Control positive (1) and negative (2), containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis. -- 1.5 mL × 2
4. Sample buffer P (5×),containing PBS, BSA detergent, preservative NaN_3 0.09%, yellow, 5× conc. -- 20 mL
5. Enzyme conjugate containing anti-human IgG; HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use. -- 15 mL
6. TMB substrate; containing 3, 3', 5, 5'-Tetramethylbenzidin, colorless. Ready to use. -- 15 mL
7. Stop solution; contains acid. Ready to use. -- 15 mL
8. Wash solution (50×), containing Tris, detergent, presearvative NaN_3 0.09%; 50× conc. -- 20 mL

Materials Required But Not Supplied

1. Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm.
2. Data reduction software
3. Multi-Channel Dispenser or repeatable pipet for 100 µL
4. Vortex mixer
5. Pipets for 10 µL, 100 µL and 1000 µL
6. Laboratory timing device
7. Distilled or deionized water
8. Measuring cylinder for 100 and 1000 mL
9. 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.

Storage

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

Specimen Collection And Preparation

1. Collect whole blood specimens using acceptable techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum or plasma by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipema should be avoided,

but does not interfere with this assay.

4. Specimens should be stored at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
6. Testing of heat-inactivated sera is not recommended.
7. Dilute the 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.

Reagent Preparation

Wash solution

Dilute the contents of one vial of the buffered wash solution concentrate (50×) with distilled or deionized water to a final volume of 1000 mL prior to use.

Sample buffer

Sample buffer P: Prior to use dilute the contents (20 mL) of one vial of sample buffer 5× concentrate with distilled or deionized water to a final volume of 100 mL.

Assay Procedure

1. Prepare enough microplate modules for all calibrators/controls and samples.
2. Pipet 100 µL of calibrators, controls and prediluted samples in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20-28°C).
4. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
5. Dispense 100 µL of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
8. Dispense 100 µL of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add 100 µL of stop solution to each well of the modules.
11. Incubate for 5 minutes at room temperature.
12. Read the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Calculation

For quantitative results plat the optical density of each calibrator versus the calibrator concentration to create a calibration cure. The concentration of samples may then be estimated from the calibration curve by interpolation.

Using data reduction software a 4-Parmeter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Interpretation Of Results

Negative: < 10 IU/mL

Positive: ≥ 10 IU/mL

Performance Characteristics

Calibration

The assay system is calibrated against the international reference preparation WHO67/183 for AMA-M2 as 100 IU/mL.

Detection Range

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

Detection Limit

Functional sensitivity was determined to be: 1IU/mL

Reproducibility

Intra-assay precision: Coefficient of ariation (CV) was calculated for each of three samples from the results of 24 determinanations 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

Interferences

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 interferrin effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Precautions

1. All reagents of this kit are strictly intended for research use only.
2. 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.

3. Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
4. Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
5. Stop Solution contains acid, classification is non-hazardous. Avoid contact with skin.
6. Controls, Calibrators, Sample Buffer and Wash Solution contain Sodium Azide (NaN_3) 0.09% as preservative. This concentration is classified as non-hazardous.
7. Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
8. 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 he opened eye with running water for at least 10 minutes. Get medical attention if necessary.
9. 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 and inert material and put the spilled material in an appropriate waste disposal.
10. Exposure controls/personal protection: Wear protective gloves of nitrile rubber or natural latex.
11. Where protective glasses. Used according to intended use no dangerous reactions known.
12. Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
13. For disposal of laboratory waste the national or regional legislation has to be observed.
14. Do not use kit components beyond their expiration dates.
15. Do not interchange kit components from different lots and products.
16. All materials must be at room temperature (20-28°C) prior to use.
17. Prepare all reagents and samples. Once started, perform the test without interruption.
18. Double determinations may be done. By this means pipetting errors may become obvious.
19. Perform the assay steps only in the order indicated.
20. Always use fresh sample dilutions.
21. Pipette all reagents and samples into the bottom of the wells.
22. To avoid carryover contamination, change the tip between samples and different kit controls.
23. Wash microwells thoroughly and remove the last droplets of Wash Solution.
24. All incubation steps must be accurately timed.
25. Do not re-use microplate wells.