Mitochondrial IgG ELISA Kit

Catalog Number KA0945

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The Mitochondrial IgG ELISA Kit is intended for the detection of IgG antibody to Mitochondrial in human serum or plasma.

Background

Mitochondrial Antibodies (MA) are directed against the E2 subunit of the pyruvate dehydrogenase enzyme complex located at the inner mitochondrial membrane (PDC-E2), the E2 subunit of the branched chain 2-oxo acid dehydrogenase complex (BCOADC-E2), the E2 subunit of the 2-oxo-glutarate dehydrogenase complex (OGDC-E2), protein X, and PDC-Ela and PDCE1. MA are found in ~95% of patients with primary biliary cirrhosis (PBC). MA in low titers are common in chronic active hepatitis and their presence does not preclude response to corticosteroids. MA disappear in about one month after orthotopic liver ransplantation (OLT) and decrease with cyclosporine treatment which might be useful in PBC. MA are found in <1% of apparently healthy Caucasoid adults. Approximately 3% of patients with PBC have scleroderma, usually of the CREST syndrome variety. In addition, MA reactive with the PDC-E2 complex are found in some patients with CREST or diffuse scleroderma, sometimes in the absence of overt liver disease. Scleroderma typically precedes PBC in those patients with both diseases.

Principle of the Assay

Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Microwells coated with Mitochondrial antigen	96 (8x12) wells
Sample Diluent (ready to use)	22 ml
Calibrator (ready to use)	1 ml
Positive Control (ready to use)	1 ml
Negative Control (ready to use)	1 ml
Enzyme conjugate (ready to use)	12 ml
TMB Substrate (ready to use)	12 ml
Stop Solution (ready to use)	12 ml
Wash concentrate 20X	25 ml

Storage Instruction

- ✓ Store the kit at 2-8°C.
- ✓ Keep microwells sealed in a dry bag with desiccants.
- ✓ The reagents are stable until expiration of the kit.
- ✓ Do not expose reagent to heat, sun, or strong light.

Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

Precautions for Use

- Precautions

✓ Potential biohazardous materials:

The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other

infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.

- ✓ This test kit is USA FDA exempt product.
- ✓ Optimal results will be obtained by strict adherence to the test protocol. Accurate and precise pipetting as following the exact time and temperature requirements prescribed are essential.
- ✓ Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- ✓ The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- ✓ Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

- Limitation

- ✓ The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
- ✓ Lipemic or hemolyzed samples may cause erroneous results.

Assay Protocol

Reagent Preparation

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26 °C).

Sample Preparation

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

Assay Procedure

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

Data Analysis

Calculation of Results

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

- Example of typical results:
  Calibrator mean OD = 0.8
  Calibrator Factor (CF) = 0.5
  Cut-off Value = 0.8 x 0.5= 0.400
  Positive control O.D. = 1.2
  Ab Index = 1.2 / 0.4 = 3
  Patient sample O.D. = 1.6
  Ab Index = 1.6 / 0.4 = 4.0

- Quality Control
  The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should be greater than 1.2.

- Interpretation
  The following is intended as a guide to interpretation of MA test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

- Antibody Index Interpretation
  <0.9 No detectable MA by ELISA.
  0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
  >1.1 Detectable MA by ELISA.

Performance Characteristics

- Sensitivity and Specificity
  113 patient sera were tested by this Mitochondrial IgG ELISA Kit and a reference ELISA method. 18 sera

were positive and 90 were negative by both methods (96% agreement). The results are summarized below:

	Mitochondrial IgG ELISA Kit		
	+	-	Total
Reference ELISA kit +	18	3	21
-	2	90	92
Total	20	93	113

- Precision

Intra-Assay

Sample	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation (%)
1	16	1.67	0.096	5.7
2	16	0.84	0.069	8.2
3	16	0.23	0.016	6.9

Inter-Assay

Sample	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation (%)
1	10	1.54	0.118	7.6
2	10	0.81	0.074	9.1
3	10	0.20	0.021	10.5

Resources

References

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Plate Layout

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