Please read this package insert carefully before use.

Capilia™ MAC Ab ELISA

INTENDED USE

To detect serum IgA antibodies to glycopeptidolipid (GPL) core derived from *Mycobacterium avium* complex (MAC).

SUMMARY AND EXPLANATION OF THE TEST

Mycobacterium avium complex (MAC) is a group of genetically related bacteria belonging to the genus Mycobacterium. MAC bacteria are common in the environment and cause infection when inhaled or swallowed.

Symptoms can be similar to those associated with tuberculosis (TB), and include fever, fatigue, and weight loss.

Because MAC is resistant to ordinary antituberculous drugs, appropriate treatment requires that MAC infections be differentiated from TB.

Capilia MAC Ab ELISA provides rapid and accurate detection of MAC by detecting anti-MAC antibodies in serum.

PRINCIPLE OF THE TEST

This product measures the anti-GPL core IgA antibodies in serum by the sandwich EIA method using microplates coated with a solid-phased antigen. The diluted serum specimen is added to a well of microplates coated with the solid-phased MAC cell wall-derived GPL core antigen to induce a reaction from the specific antibodies present in the serum. After the well is washed, peroxidase-labeled anti-human IgA antibodies are added to form an immune complex with the IgA specific antibodies in the serum bonded with the solid-phased GPL core antigen.

After the well is washed, a chromogen solution is added to produce color, and the stop solution is then added to stop the coloration reaction. After the reaction is stopped, absorbance at 450 nm is measured and the anti-GPL core IgA antibody concentration (The concentration is expressed as U/mL〔U: a unit set by our company〕) for the tested serum is determined from the calibration curve established on the basis of the absorbance of the calibrator. The result is compared with the cut-off value to determine whether it is positive or negative.

REAGENTS AND MATERIALS PROVIDED

1. Microplates coated with a solid-phased antigen
   Glycopeptidolipid (GPL) core antigen
2. Enzyme-labeled antibody solution
   Peroxidase-labeled anti-human IgA antibody (mouse)
3. Chromogen solution
   3,3',5,5'-Tetramethylbenzidine (TMB)
4. Specimen diluent
5. Stop solution
6. Wash solution (×5)
7. Cover for Microplates coated with a solid-phased antigen

MATERIALS REQUIRED BUT SOLD SEPARATELY

Capilia MAC Ab ELISA Calibrator (5 conc. × 0.25 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micropipettes (those for 10 µL, 50 µL, 100 µL and 400 µL volumes)
2. Sequential dispensing pipettes or micropipettes for 50 µL and 100 µL volumes
3. Purified water
4. Incubator (when the room temperature is not higher than 20°C, the incubator should be set at 20°C to 30°C.
5. Microplate washer for ELISA, or sequential dispensing pipettes for 300 µL volume and aspirators.
6. Microplate reader for ELISA (450nm measurement filter)
7. Timer
8. Sodium hypochlorite solution (valid chlorine concentration: not less than 1,000 ppm (0.1%) or a 2% glutaraldehyde solution.
9. Data processing computer or graph paper

WARNING AND PRECAUTIONS

1. Precautions for handling (hazard prevention)

1) Infection with pathogenic microbes, such as HBV, HCV, HIV, etc. may occur from specimens. Use gloves so as not to touch specimens with the bare hands and exercise caution in handling to prevent infection.
2) Do not pipette with the mouth.
3) If the reagent gets into your eyes or mouth, or comes into contact with your skin, immediately flush with a large quantity of water as an emergency measure. If you still feel some abnormality, see a doctor for treatment.
4) The stop solution contains 1 M sulfuric acid. When using, exercise caution so that the solution does not come into contact with eyes, skin or clothes.
5) If you spill the reagent, dilute it with water and wipe it away. If you spill the specimen, sprinkle 80% ethanol and wipe it away.

2. Precaution when using

1) Do not reuse a microplate coated with a solid-phased antigen.
2) Do not use any reagents beyond the expiration date.
3) Do not use reagents with a different lot number than that on the outer package.
4) If any anomaly is found in the appearance of the reagent, do not use it.
5) Store this product according to the storage instructions.
6) If the reagent has been frozen by mistake, do not use it. Correct results may not be obtained, because of a change in quality after freezing.

3. Precautions for disposal

1) All specimens and apparatus that have been in contact with specimens should be treated according to the following procedures or to the infectious medical waste disposal manual at each institution.
   ① Perform autoclave sterilization at 121°C for at least 20 minutes.
   ② Soak in 2% glutaraldehyde for at least 1 hour.
   ③ If combustible, incinerate.
2) **The stop solution contains 1 M sulfuric acid. When you dispose of the stop solution, microplate, etc., do not mix the solution with sodium hypochlorite.**
3) When reagents, apparatus, etc. are disposed of, they should be treated in accordance with the laws and regulations concerning medical waste disposal and water pollution control.

4. Other precautions

This product is a test kit for detecting anti-GPL core IgA antibodies in serum. A definite diagnosis should be made by an attending physician, in combination with clinical symptoms, diagnostic imaging, culture method, and other test results.

STORAGE CONDITIONS

Storage : Keep in a dark cool place at 2°C to 8°C. **DO NOT FREEZE.**
Keep away from direct sunlight.
Do not use any reagents or materials beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

1. Precautions concerning specimens

1) Use serum for measurement with this product.
2) Specimens should be prepared and measured rapidly after drawing blood. For long-term storage, tightly seal the serum sample vial and store it at not higher than -20°C. Avoid repeated freezing and thawing.
3) Specimens diluted with the specimen diluent should be used for the measurement on that day.
4) If specimens contain insoluble or viscous matters, remove them by centrifugation or filtration before testing. Such matters may interrupt a reaction.

2. Preparation of reagent

1) Microplates coated with a solid-phased antigen
   The microplate is ready for use after being acclimatized to room temperature. Determine the number of microplate needed for assay and add microplate to attached the frame. Once opened, the unused microplate coated with a solid-phased antigen should be kept in the zip-up aluminum pouch at 2°C to 8°C and used as soon as possible.
2) The enzyme-labeled antibody solution
   The solution is ready for use after being acclimatized to room temperature.
3) The chromogen solution
   The solution is ready for use after being acclimatized to room temperature.
4) The specimen diluent
   The diluent is ready for use after being acclimatized to room temperature.
5) The stop solution
   The solution is ready for use after being acclimatized to room temperature.
6) The wash solution
   The solution is used after being acclimatized to room temperature and diluted 5 times with purified water.

TEST PROCEDURE

1. Sample preparation

Add 250 µL of purified water to each of the 5 different concentrations of the calibrators [sold separately: Capilia MAC Ab ELISA Calibrator (5 conc. × 0.25 mL)]. Gently stir and mix by inverting. Before use, confirm that the solution is completely dissolved.

The human serum to be tested should be diluted 41 times with the specimen diluent.

Example of dilution: Add 10 µL of the human serum to be tested to 400 µL of the specimen diluent and mix them well.

2. Test procedures

1) Primary reaction
   All samples are tested in duplicate. Separately add 50 µL each of the 5 different concentrations of the calibrators (sold separately: to be used after being dissolved in 250 µL of purified water) and 50 µL of the 41-fold diluted serum to be tested to each of the two wells. For the reagent blank, a specimen diluent is used. Cover the microplates with the dedicated cover and incubate at room temperature for 1 hour.
2) Washing
   Wash the wells 4 times with 300 µL of the wash solution per well each time.
3) Secondary reaction
   Add 50 µL of the enzyme-labeled antibody solution to each well. Cover the microplates with the dedicated cover and incubate at room temperature for 1 hour.
4) Washing
   Wash the wells 4 times with 300 µL of the wash solution per well each time.
5) Coloration
   Add 100 µL of the chromogen solution to each well. Cover the microplates with the dedicated cover and incubate at room temperature for 20 minutes.
6) Stopping the reaction
   Add 100 µL of the stop solution to each well.
7) Optical density (OD) measurement
   Measure the absorbance at 450 nm.

When you use a microplate reader for ELISA that does not allow selecting the 450 nm test wavelength, select a wavelength within the range between 420 and 470 nm.

Note:

1) Store this product at 2°C to 8°C and acclimatize it to room temperature before use.
2) For dilution of the specimen, use the specimen diluent attached to the product. If a diluent other than the attached is used, the correct result may not be obtained.
3) Measure the sample in duplicate, and establish the calibration curve for each measurement.
4) The wash solution (×5) should be mixed by inverting and stirred well before use.
5) Caution should be exercised so as not to dry out or scratch the well during measurement procedures.
6) Wipe away droplets of the solution or dirt on the underside of the well before measuring the absorbance of the well.
7) The unused microplate coated with a solid-phased antigen should be sealed in the zip-up aluminum pouch and stored under refrigeration.
8) Separate pipette tips should be used for each sample and reagent. Do not use the same pipette tip for different materials.
9) To obtain reproducible results, pipetting should be carried out precisely and the well should be washed thoroughly.

READING THE RESULTS

To prepare a calibration curve, the calibrators of 5 different concentrations (sold separately) are used.

Plot the points representing the anti-GPL core IgA antibody concentrations (U/mL) on the X axis and the absorbances (ΔOD mABS) on the Y axis and connect each of them by straight lines to prepare a calibration curve. Using this calibration curve, the antibody concentration corresponding to the absorbance is calculated. The result is compared with the cut-off value to determine the reading.

<Reading of the Results>

Reading	Reference cut-off value
Negative	Less than 0.7 U/mL
Positive	More than 0.7 U/mL

LIMITATIONS

1. This product is a test kit for detecting anti-GPL core IgA antibodies in serum. A definite diagnosis should be made by an attending physician, in combination with clinical symptoms, diagnostic imaging, culture method, and other test results.
2. Please use this product following the operational method described in this package insert. We cannot guarantee results obtained from any other operations and for any other purposes that are not described in the package insert.

PERFORMANCE CHARACTERISITICS

1. Clinical data

1) Identification of cut-off value

Receiver operating characteristic (ROC) analysis was conducted in 76 cases of healthy males and females in their 20s to 70s who were unlikely to be infected with non-tuberculous mycobacteria, 37 cases who were clinically diagnosed as having pulmonary TB, 43 cases who were diagnosed as having other chest diseases, and 70 cases who were clinically diagnosed as having MAC disease. When placing emphasis on specificity and the cut-off value is set at 0.7 U/mL, the true positive ratio accounted for 84.3% and the true negative ratio accounted for 100%.

2) The positive ratio of anti-GPL core IgA antibodies in patients with MAC disease

In the test of the serum samples from the population of patients who were diagnosed as having MAC disease in the first medical examination that had been untreated, the positive ratio of anti-GPL core IgA antibodies on the basis of the cut-off value of 0.7 U/mL was 84.3% (59 of 70 patients).

3) The true negative ratio in differential diagnosis with other diseases

	The true negative ratio (number of patients tested negative/total number of patients).
Pulmonary tuberculosis	100% (37/37)
Other chest diseases [Note]	100% (43/43)

[Note] Other chest diseases : lung cancer, interstitial pneumonia, chronic obstructive pulmonary disease (COPD), and sarcoidosis.

・ In the case of an infection with *M. abscessus*, *M. scrofulaceum*, *M. fortuitum* or *M. chelonae*, any of which has GPL, a positive result may be obtained.

2. Sensitivity (Detection limit)

The minimum detection sensitivity for anti-GPL core IgA antibodies in serum obtained by this product is 0.5 U/mL.

3. Reproducibility

When the same specimen was measured 5 times simultaneously, the coefficient of variation (CV) was less than 10%.

INTERFERING SUBSTANCES

In this product, coexisting substances were found to have no effect within the concentrations given below.

Hemoglobin released by hemolysis	523 mg/dL
Free bilirubin	19.4 mg/dL
Conjugated bilirubin	20.9 mg/dL
Chyle serum	2,800 formazine turbidity unit (FTU)
Rheumatoid factor	500 IU/mL (IgM-RF)

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ASSAY PROCEDURE SUMMARY

Reagents, Calibrators and Sample Preparation

- Wash solution: Dilute wash solution 1:4 with purified water
- Calibrator: Add 250 µL of purified water to each of the 5 different concentrations of the calibrators
- Sample: The human serum should be diluted 41 times with the specimen diluent

Assay procedure

Add 50 µL of calibrator or sample to each well

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Cover the microplates with the dedicated cover and incubate at room temperature for 1 hour
Aspirate and wash 4 times with 300 µL of the wash solution

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Add 50 µL of the enzyme-labeled antibody solution to each well

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Cover the microplates with the dedicated cover and incubate at room temperature for 1 hour
Aspirate and wash 4 times with 300 µL of the wash solution

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Add 100 µL of the chromogen solution to each well

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Cover the microplates with the dedicated cover and incubate at room temperature for 20 minutes

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Add 100 µL of the stop solution to each well and measure the absorbance at 450 nm

INQUIRES

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GLOSSARY OF SYMBOLS

CE	CE Marking (European directive 98/79/EC on *in vitro* diagnostic medical devices)	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device		Manufacturer/Manufactured by
2℃ 8℃	Temperature limitation		Consult instructions for use
	Use by YYYY-MM		Caution, consult accompanying documents.
LOT	Batch code		Keep away from sunlight
REF	Catalog number		Fragile, handle with care
Σ n	Contents sufficient for <n> tests		