

For Research Use Only.
Not for use in diagnostic procedures.

Anti-MDA5 ELISA Kit

Cat No. 7874R

INTENDED USE

Anti-MDA5 ELISA Kit is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti-melanoma differentiation associated gene 5 protein (MDA5) antibodies in human serum. **Anti-MDA5 ELISA Kit is intended for research use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

MDA5, also known as CADM-140 is one of the retinoic acid inducible gene I (RIG-I) family proteins and involved in innate immune defense against viruses, cellular growth suppression, and apoptosis. And these responses also could trigger the autoimmune response to MDA5.

The Anti-MDA5 ELISA Kit is for measuring anti-MDA5 antibody specifically with high sensitivity in ELISA.

PRINCIPLE

The Anti-MDA5 ELISA Kit is an ELISA that measures anti-MDA5 antibodies in human serum sample. Calibrators and samples are added to the Microwells coated with recombinant human MDA5 antigen. During sample incubation, anti-MDA5 antibodies will react with the immobilized antigen. After washing to remove any unbound serum proteins, horseradish peroxidase conjugated anti-human IgG is added and incubated (Conjugate incubation). After 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 test result is determined photometrically by measuring the absorbance and plotting the results.

BRIEF ASSAY PROCEDURE

<Sample incubation> (20-30°C) 30 min.	Add 100 µL of diluted sample (1:101) to each well of the Microwell plate ↓ Wash ↓
<Conjugate incubation> (20-30°C) 30 min.	Add 100 µL of Conjugate to each well ↓ Wash ↓
<Substrate incubation> (20-30°C) 15 min.	Add 100 µL of Substrate to each well ↓ Add 100 µL of Stop solution to each well ↓ Read absorbance (450 nm) ↓ Calculate Unit value (U/mL) ↓ Interpret the result

REAGENTS AND STORAGE

1. MDA5 Microwell strips

96 wells (12 × 8 wells) coated with recombinant MDA5 antigen. The breakaway strips are packed in a strip holder and sealed in a ziplock pouch with a desiccant.

2. Calibrator 1 (0 U/mL)

One vial of 1.5 mL, containing 0.09% sodium azide. Ready to use.

3. Calibrator 2 (100 U/mL)

One vial of 1.5 mL, containing anti-MDA5 antibody and 0.09% sodium azide. Ready to use.

4. Conjugate

One vial of 15 mL, containing horseradish peroxidase conjugated goat anti-human IgG antibody and ProClin 150. Ready to use.

5. Assay diluent

Two vials of 50 mL each, containing PBS, Tween 20 and 0.09% sodium azide. Ready to use.

6. Wash buffer (20×)

One vial of 50 mL, containing PBS and Tween 20, as a 20× concentrate.

7. Substrate

One vial of 20 mL, containing 3,3',5,5'-tetramethylbenzidine dihydrochloride and hydrogen peroxidase (TMB/H₂O₂).

8. Stop solution

One vial of 20 mL, containing 0.25 mol/L sulfuric acid. Ready to use.

9. High control

One vial of 1.5 mL, containing anti-MDA5 antibody and 0.09% sodium azide. Ready to use.

10. Low control

One vial of 1.5 mL, containing human serum and 0.09% sodium azide. Ready to use.

Unopened kit components are stable until the labeled expiration date at 2-8°C.

PRECAUTIONS

1. This product is for research use only.
2. Do not use the kit components beyond the stated expiration dates.
3. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
4. Avoid contact of the reagents with eyes, skin and clothing. Reagents on skin must be washed away with plenty of water. TMB is irritating and the Stop solution consists of 0.25 mol/L sulfuric acid, which is a poison and is corrosive.
5. Low control contains human serum, in which HBs antigen, HCV antibody, HIV-1 and HIV-2 antibodies have 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.
6. Calibrator 1, Calibrator 2, High control, Low 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.

7. MDA5 Microwell strips, Calibrator 1, Calibrator 2, Conjugate, Assay diluent, High control and Low control contain materials of animal origin, taken from non-infected animals. These components, however, should be treated as potential biohazards in use and for disposal.
8. Do not mix reagents from other kits.
9. All reagents must be brought to room temperature (20-30°C) before starting the assay.
10. Do not expose the kit to direct sunlight during the test and storage.
11. Avoid microbial and cross contamination of reagents or samples. Contamination of the TMB substrate solution with Conjugate or other oxidants will cause the solution to change color prematurely.
12. Incubation temperatures above or below normal room temperature (20-30°C), shorter or longer incubation times and inaccurate dilutions may give erroneous results.
13. The wells must be properly rinsed with Wash solution to avoid false results.
14. Carefully pipette each sample and reagent to avoid cross contamination between Microwells, avoid foaming.
15. All unused Microwell strips should be returned to the ziplock pouch, which must be carefully resealed to avoid moisture absorption.
Do not allow wells to become dry during an assay procedure.
16. Wash buffer may become turbid at 2-8°C.
Dissolve the Wash buffer completely when preparing the Wash solution.
17. Materials used for the test should be disposed or treated as shown below.
Soak in 2% final conc. glutaraldehyde solution for more than 1 hour, soak in 0.1% sodium hypochlorite solution (available chlorine: approx. 1,000 ppm) for more than 1 hour or autoclave at 121°C for more than 20 minutes.
18. All equipment should be properly maintained according to the manufacturer's instructions.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader (wavelength: 450 nm)
- Multichannel micropipette (e.g. 100 µL - 300 µL)
- Single channel pipette (e.g. 10 µL & 100 µL)
- Autowasher or washing bottle
- Deionized or distilled water
- One liter graduated cylinder for preparation of Wash solution
- Test tubes for sample dilutions (e.g. 1,000 µL)
- Disposable pipette tips
- Paper towels
- Microplate cover

PROCEDURE

■ PREPARATION OF REAGENTS

1. Bring all assay materials to room temperature (20-30°C) prior to use.
2. Microwell strips: Remove required Microwell strips from the ziplock pouch and place them in the frame. Promptly return unused strips to the refrigerated storage.

3. Wash solution: Prepare 1:20 dilution of the Wash buffer prior to use (ex. add 50 mL of Wash buffer to 950 mL of distilled water). The diluted Wash solution is stable for 2 weeks at 2-8°C.
4. Do not dilute Calibrator 1, Calibrator 2, High control, Low control, Conjugate, Assay diluent, Substrate and Stop solution. These reagents are ready-to-use.

■ PREPARATION OF SAMPLES

1. Use fresh human sera. Samples should be tested as soon as possible after collection. If storage is needed after the test, they should be stored at -20°C or lower. Do not repeat freezing and thawing.
2. Dilute each human serum 1:101 by adding 10 µL of serum to 1 mL of Assay diluent.
 - * Dilute samples for each assay because they cannot be stored.
 - * Assay diluent may form precipitates, which does not cause inconsistent results.

■ ASSAY PROCEDURE

STEP 1. (SAMPLE INCUBATION)

Transfer 100 µL of Calibrator 1, Calibrator 2, High control, Low control and each diluted sample, with a multichannel micropipette, into the appropriate Microwells of the antigen test plate. (Do not dilute Calibrator 1, Calibrator 2, High control and Low control.)

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

Cover the wells with a microplate cover and incubate for 30 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)

Add 100 µL of the Conjugate to each well with a multichannel micropipette.

- * Do not return the Conjugate once taken out of vial.

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

STEP 4. (WASHING)

Wash the microplate as described in STEP 2.

STEP 5. (SUBSTRATE INCUBATION)

Add 100 µL of the Substrate to each well with a multichannel micropipette.

- * Do not return the Substrate once taken out of vial.

Cover the wells with the microplate cover and incubate for 15 minutes at room temperature (20-30°C).

STEP 6. (STOP REACTION)

Add 100 µL of the Stop solution to each well with a multichannel micropipette.

■ READING

Read the absorbance of each well at 450 nm.

- * Reading should be done within 20 minutes after stopping the reaction.
- * Before reading the plate, 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.

■ CALCULATION OF RESULT

$$\text{Unit value (U/mL)} = \frac{(A_{450} < \text{Sample} > - A_{450} < \text{Calibrator 1} >)}{(A_{450} < \text{Calibrator 2} > - A_{450} < \text{Calibrator 1} >)} \times 100$$

- * A_{450} is abbreviation of absorbance value at 450 nm.
- * An international reference material for anti-MDA5 antibodies is not available. The assay is calibrated in relative arbitrary units.

■ QUALITY CONTROL

Each assay result should meet the following criteria.

A_{450} of Calibrator 1: ≤ 0.100

A_{450} of Calibrator 2: ≥ 0.500

High control and the Low control unit value: As described in each label

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

Before repeating the test, check the following points.

- Incubation Temperature
- Incubation Time
- Washing

PERFORMANCE CHARACTERISTICS

■ SENSITIVITY

A_{450} of Calibrator 1 (0 U/mL) shall be ≤ 0.100

A_{450} of Calibrator 2 (100 U/mL) shall be ≥ 0.500

■ SPECIFICITY

When measuring control samples of known concentrations, each value shall be within $\pm 20\%$ of known concentration.

■ PRECISION

When measuring control samples of known concentrations sixfold simultaneously, CV% of each sample shall be within 15%.

■ ASSAY RANGE

The assay range of this kit is from 5 to 150 U/mL.

■ LIMITS OF DETECTION

Unit value = 5 U/mL

■ INTERFERING SUBSTANCES

Hemoglobin (up to 487.0 mg/dL), Bilirubin C (up to 20.9 mg/dL), Bilirubin F (up to 19.5 mg/dL), chyle (up to 1410.0 FTU) or Rheumatoid factor (up to 550.0 IU/mL) are not affective on the assay result, but avoid using highly hemolysed samples or highly lipemic samples.

REFERENCES

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3. Nakashima R, et al. The RIG-I-like receptor IFIH1/MDA5 is a dermatomyositis-specific autoantigen identified by the anti-CADM-140 antibody. *Rheumatology (Oxford).* 2010;49(3): 433-440.

*Manufactured by:

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

SUMITOMO FUDOSAN SHIBADAIMON NICHOME BLDG.

2-11-8 Shibadaimon, Minato-ku, Tokyo 105-0012 Japan

Tel: +81-3-6684-6860

URL: <http://www.mbl.co.jp/>

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