Human anti-mutated citrullinated vimentin (MCV) antibody ELISA Kit

Catalog No. CSB-E09565h

(96 tests)

- This immunoassay kit allows for the in vitro semi-quantitative determination of **human anti-MCV antibody** concentrations in **serum, plasma**.
- **Expiration date   six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with purified MCV antigen. Samples are then added to the appropriate microtiter plate wells and incubated. Then add Horseradish Peroxidase (HRP)-conjugated anti-human IgG to each well and incubate. Finally, a substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of human anti-MCV antibody in the samples is then determined by comparing the O.D. of the samples to the standard curve.

DETECTION RANGE

5U/ml-400U/ml. The standard curve concentrations used for the ELISA's were 400 U/ml，100 U/ml，25 U/ml，10 U/ml，5U/ml

SPECIFICITY

This assay recognizes human anti-MCV antibody. No significant cross-reactivity or interference was observed.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Sample Diluent	1 x 20 ml
HRP-conjugate	1 x 10 ml
Standard(S1-S5)	5 x 1ml
Wash Buffer (25×concentrate)	1 x 20 ml
Substrate A	1 x 5 ml
Substrate B	1 x 5 ml
Stop Solution	1 x 5 ml
Positive Control	1 x 1ml
Negative Control	1 x 1ml

Standard	S1	S2	S3	S4	S5
Concentration(U/ml)	5	10	25	100	400

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.

2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARTION

Sample requires a 101-fold dilution into Sample Diluent. The suggested 101-fold dilution can be achieved by adding 2µl sample to 200µl of Sample Diluent.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards and controls be assayed in duplicate.

1. Set a Blank well without any solution.

2. Add 100µl of **Positive Control**, **Negative Control**, **Standard (S1-S5)** and **diluted Sample** per well. Cover with the adhesive strip. Incubate for 30 minutes at 37°C.

3. Aspirate each well and wash, repeating the process for a total of **five** washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100µl of **HRP-conjugate** to each well (Not to Blank well). Incubate for 30 minutes at 37°C.

5. Wash plate **five** times as before.

6. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well. Incubate for 10 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

7. Add 50µl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Determine the optical density of each well within 20 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average optical density of Blank. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human anti-MCV antibody concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

While Concentration$_{sample}$≥25U/ml : Positive

While Concentration$_{sample}$＜25U/ml : Negative

Quality Control

In order to ensure the accuracy of results, negative control and positive control should be detected simultaneously with samples. OD values of negative control should be less than 0.100 and OD values of positive control should be greater than 1.000.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.

- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.

- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.