Measles Virus IgM (MV IgM) ELISA Kit

Catalog No.: abx364863

Size: 96T

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 4°C for up to 6 months.

Application: For quantitative detection of MV IgM in Serum and plasma.

Introduction: Measles virus is a single-stranded, negative-sense, enveloped (non-segmented) RNA virus of the genus Morbillivirus within the family Paramyxoviridae. Humans are the natural hosts of the virus; no animal reservoirs are known to exist. The measles virus is the cause of measles, an infection of the respiratory system. The measles virus has two envelope glycoproteins on the viral surface—hemagglutinin (H) and membrane fusion protein (F). These proteins are responsible for host cell binding and invasion. Three receptors for the H protein have been identified to date: complement regulatory molecule CD46, the signaling lymphocyte activation molecule (SLAM) and the cell adhesion molecule Nectin-4.

Principle of the Assay

This kit is based on indirect enzyme-linked immuno-sorbent assay technology. The 96-well plate is pre-coated with the target antigen. Samples are added to the wells and incubated, then washed with wash buffer. Samples that contain MV IgM will bind to the pre-coated antigen to form an antigen-antibody complex. Next, HRP-conjugated detection antibody is added to the wells and incubated, then washed. Any bound MV IgM will TMB substrate is added, which is catalyzed by HRP to produce a blue colour product that changes into yellow after adding stop solution. The intensity of the color yellow is proportional to the amount of MV IgM bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of MV IgM can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Positive control: 1 ml
3. Negative control: 1 ml
4. MV antigen: 6 ml
5. HRP-conjugated antibody: 6 ml
6. Sample diluent buffer: 12 ml
7. Concentrated Wash buffer (20X): 50 ml
8. TMB substrate A: 6 ml
9. TMB substrate B: 6 ml
10. Stop solution: 6 ml
11. Plate Sealer: 3
12. Hermetic bag: 1

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. Distilled or deionized water
7. 1.5 ml tubes to prepare standard/sample dilutions
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 25 ml of concentrated wash buffer into 475 ml of distilled water).

B. Assay Procedure

Equilibrate the kit components and samples to room temperature for at least 30 minutes prior to use.

1. Determine the number of wells to be used. Any strips that are not being used should be kept dry and stored at 4°C.
2. Set up three Negative Control wells with 100 µl of Negative Control per well. Set up three Positive Control wells with 100 µl of Positive Control per well. Set up two blank wells with no solution.
3. In a separate tube, dilute samples 1:10. Mix thoroughly. Add 100 µl of diluted samples to each sample well.
4. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 60 minutes.
5. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300µL) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
6. Add 50 µl of MV antigen to each well (except the blank well). Gently tap the plate to mix thoroughly. Then add 50 µl of HRP-conjugate to each well (except the blank well). Gently tap the plate to mix thoroughly. Cover the plate with the plate sealer and incubate at 37°C for 30 minutes.
7. Remove the cover and discard the liquid. Wash the plate 5 times as explained in step 5.
8. Add 50 µl of TMB substrate A and 50 µl of TMB substrate B to each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 10 minutes in the dark.
9. Remove the cover and add 50 µl of Stop solution to each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.

10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm within 10 minutes of adding the stop solution.

C. Analysis

1. **Calculations:**
   Mean absobance of the positive control should be ≥ 0.60.
   Mean absobance of the negative control should be ≤ 0.10.
   CUT OFF value (Negative control < 0.05) = 0.05 + 0.10 = 0.15
   CUT OFF value (Negative control ≥ 0.05) = Negative control + 0.10

2. **Interpretation of results:**
   If the postive control value is ≥ 0.60, negative control value is ≤ 0.10, and blank value is ≤ 0.08, the test is valid, otherwise, the test is invalid.
   Samples:
   If O.D. of samples < CUT OFF, the test samples are considered negative.
   If O.D. of samples ≥ CUT OFF, the test samples are considered positive.
   Precision:
   Intra-variable coefficient (CV%) ≤ 15%

C. Precautions

1. Ensure that the plate remains dry until starting the assay.
2. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. Do not use components from a different kit or expired ones.
11. The TMB Substrate B solution is easily contaminated; work under sterile conditions when handling the TMB substrate B solution. The TMB Substrate B solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.