Human Mumps Virus (MuV) IgM ELISA Kit

Catalog No.: abx364942

Size: 96T

Range: Qualitative

Sensitivity: Qualitative

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

Application: For quantitative detection of MuV in Human Serum

Introduction: Mumps is a viral disease caused by the mumps virus. Initial signs and symptoms often include fever, muscle pain, headache, poor appetite, and feeling generally unwell. This is then usually followed by painful swelling of one or both parotid salivary glands. Symptoms typically occur 16 to 18 days after exposure and resolve after seven to ten days. Symptoms are often more severe in adults than in children. About a third of people have mild or no symptoms. Complications may include meningitis (15 percent), pancreatitis (four percent), inflammation of the heart, permanent deafness, and testicular inflammation which uncommonly results in infertility.

Principle of the Assay

This kit is based on enzyme-linked immuno-sorbent assay technology. An antibody specific to Human IgM is pre-coated onto a 96-well plate. The controls and test samples are added to the wells and washed with wash buffer. HRP-conjugated MuV antigen is added and will attach to any available MuV binding sites. After washing, TMB substrate is used to visualise HRP activity. TMB is catalysed by HRP to produce a blue colour product that changes to yellow after adding stop solution. The intensity of the color yellow is proportional to the MuV amount bound on the plate. The O.D. absorbance is measured spectrophotometrically in a microplate reader, and then the concentration of MuV 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. HRP Conjugate: 12 ml
5. Sample Diluent buffer: 12 ml
6. Concentrated Wash buffer (20X): 50 ml
7. TMB Substrate A: 6 ml
8. TMB Substrate B: 6 ml
9. Stop solution: 6 ml
10. Plate sealer: 3
11. Hermetic bag: 1

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm or 450/630 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

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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.

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.

» Heat inactivated samples are not recommended as they can degrade the antibodies used in this kit.

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. (Blank wells are not required if using dual wavelength detection at 450/630 nm.)

3. In a separate tube, dilute samples 1:10 (i.e. add 100 µl of Sample Diluent buffer to 10 µl of sample). 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 30 minutes.

5. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with 1X Wash buffer (approximately 350 µl) 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, then invert the plate and blot it against clean absorbent paper towels.

6. Add 100 µl of HRP Conjugate to each well (except the blank well). Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. 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 15 minutes in the dark.

9. Remove the cover and add 50 µl of Stop solution to each well. There should be a colour change to yellow. Gently tap the plate to ensure thorough mixing.

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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 (or at dual wavelength 450/630 nm) within 10 minutes of adding the stop solution.

C. Analysis

1. **Calculations:**
   Mean absorbance of the positive control should be ≥ 0.8.
   Mean absorbance of the negative control should be ≤ 0.1.
   Mean absorbance of the blank wells should be ≤ 0.08.
   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 ≥ 0.8, the blank well value is ≤ 0.08, and the negative control value is ≤ 0.1, 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:
   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 colourless or very light yellow in appearance. Aspirate the dosage needed with sterilised tips and do not dump the residual solution back into the vial.

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