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Product Information

Mumps IgM ELISA

Catalog Number **SE120094**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Infection with mumps virus causes fever, headache, and swelling and tenderness of the salivary glands. Most adults born before 1957 have been infected naturally and are probably immune. Mumps can occur in unimmunized children, or adolescents and young adults who graduated from school prior to the law requiring mumps immunization. About 1/3 of people have no symptoms. The first symptoms usually appear 16–18 days after exposure. It begins with fever and pain upon opening the mouth or eating. Possible complications include meningitis (swelling of the covering of the brain and spinal cord), encephalitis (swelling of the brain), deafness, and in adult males, swelling of the testicles. The virus may cause a miscarriage if a woman becomes infected during the first three months of pregnancy. Mumps IgM antibodies are present in serum of 72% of patients by day 2 of clinical illness and in essentially all patients after day 5. A significant increase in titer of mumps IgG is found in over 90% of paired acute and convalescent mumps sera in which mumps IgM antibodies can also be found. Increases in mumps antibody titers in paired acute and convalescent sera are valuable for confirmation of acute infection even in the presence of specific IgM antibodies because 50% of patients still have elevated levels of reactive IgM, 5 or more months after clinical mumps. In mumps meningitis, the Mumps IgG Antibody Index is increased in about 83% of patients and the Mumps IgM Antibody Index is increased in about 67% of those with detectable IgM in the CSF.

The Mumps IgM ELISA kit is an enzyme linked immunosorbent Assay (ELISA) for the detection of IgM class antibodies to mumps in human serum or plasma. Diluted serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess Enzyme Conjugate is washed off and substrate is added. The plate is incubated to allow the oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

Components

Materials Provided	96 Tests
Microwells coated with Mumps antigen	12 x 8 x 1
Sample Diluent: 1 bottle (ready to use)	22 mL
Calibrator: 1 Vial (ready to use)	1 mL
Positive Control: 1 vial (ready to use)	1 mL
Negative Control: 1 vial (ready to use)	1 mL
Enzyme conjugate: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
Wash concentrate 20x: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph Paper

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Sample Preparation
1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

20x Wash Buffer Concentrate
Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

The test run may be considered valid provided the following criteria are met:

1. If the O.D. of the Calibrator is >0.250.
2. The Ab index for Negative control should be <0.9.
3. The Ab index for Positive control should be >1.2.

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 21-fold dilution of test samples, by adding 10 µL of the sample to 200 µL of Sample Diluent. Mix well.
3. Dispense 100 µL of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µL of Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µL of 1x Wash Buffer. Blot on absorbent paper or paper towel.
5. Dispense 100 µL of Enzyme Conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µL of 1x Wash buffer. Blot on absorbent paper or paper towel.
7. Dispense 100 µL of TMB Substrate and incubate for 10 minutes at room temperature.
8. Add 100 µL of Stop Solution.
9. Read O.D. at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600–650 nm.

Results

Calculations

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5 = 0.400
Positive control O.D. = 1.2
Ab Index = 1.2/0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6/0.4 = 4.0

Notes: To enhance sensitivity and specificity of this IgM test, the provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and shows no interference with test results. It can be removed by centrifugation.

In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.

Lipemic or hemolyzed samples may cause erroneous results.

Interpretation

The following is intended as a guide to interpretation of Mumps IgM antibody index (Ab Index) test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

<0.9 – No detectable antibody to mumps IgM by ELISA
0.9–1.1 – Borderline positive. Follow-up testing is recommend if clinically indicated.
>1.1 – Detectable antibody to mumps IgM by ELISA

References

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3. Nigro, G. et al., Determination of vaccine-induced and naturally acquired class-specific mumps antibodies by two indirect enzyme-linked immunosorbent assays. J. Virol. Methods, 1986; 13(2):91-106.
4. Harmsen, T. et al., Comparison of a neutralization enzyme immunoassay and an enzyme-linked immunosorbent assay for evaluation of immune status of children vaccinated for mumps. J. Clin. Microbiol., 1992;30(8):2139-44.
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6. Johnson, C.E. et al., Antibody persistence after primary measles-mumps-rubella vaccine and response to a second dose given at four to six vs. eleven to thirteen years. Pediatr. Infect. Dis. J., 1996;15(8):687-92.

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