Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Mumps IgM ELISA Kit

10/18

(Catalog # E4668-100, 96 assays; Store at 2-8^{0}C)

I. Introduction:

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 to 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 by ELISA 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 by ELISA 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.

II. Application:

Detection of IgM antibody to Mumps

III. Sample Type:

Human serum or plasma

IV. Kit Contents:

Components	E4668-100	Part No.
Microplate	12 strips x 8 wells	E4668-100-1
Sample Diluent	22 ml	E4668-100-2
Calibrator	1 ml	E4668-100-3
Positive Control	1 ml	E4668-100-4
Negative Control	1 ml	E4668-100-5
Enzyme conjugate	12 ml	E4668-100-6
TMB Substrate	12 ml	E4668-100-7
Stop Solution	12 ml	E4668-100-8
Wash Buffer (20X)	25 ml	E4668-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VI. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{0}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Buffer:** 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).

VII. Warning & Precautions:

- Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories."
- 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.
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

VIII. Sample Preparation and Storage:

Collect blood specimens & separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 7 days. Store frozen at (-20°C) for up to six month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well.

IX. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 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 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 absorbance 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 absorbance 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 min. A dual wavelength is recommended with reference filter of 600-650 nm.

X. Calculation

Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF). 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

QUALITY CONTROL
The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

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

ANTIBODY INDEX INTERPRETATION

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

LIMITATIONS OF THE TEST
1. To enhance sensitivity and specificity of this IgM test 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 has shown no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
4. Lipemic or hemolyzed samples may cause erroneous results.

Sensitivity and Specificity
129 patient sera were tested by this Mumps IgM ELISA and a reference ELISA method. 19 sera were positive and 105 were negative by both methods (96% agreement). The results are summarized below:

		Mumps IgM ELISA		
		+	–	Total
Reference ELISA Kit	+	19	3	22
	–	2	105	107
	Total	21	108	129

Precision
Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.47	0.07	4.76
2	16	1.02	0.05	4.90
3	16	0.25	0.02	8.00

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.38	0.11	7.97
2	10	0.91	0.09	9.89
3	10	0.26	0.03	11.53

XI. RELATED PRODUCTS:

- Gentamicin (serum/urine) ELISA Kit (K4315)
- QuickDetect™ IgM (Human) ELISA Kit (E4479)
- Amylase Activity Colorimetric Assay Kit (K711)
- Mycoplasma DNA Kit (K1416)
- QuickDetect™ IgG (Human) ELISA Kit (E4475)

FOR RESEARCH USE ONLY! Not to be used on humans.