

Product Information

Mumps IgG ELISA kit

Catalog Number: EA100953

Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Mumps IgG ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to Mumps in human serum or plasma.

Background

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.

Principle of the Test

Diluted patient serum is added to wells coated with purified antigen. IgG 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 hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Mumps antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml

3. Calibrator: 1 Vial (ready to use)	1ml
4. Positive Control: 1 vial (ready to use)	1ml
5. Negative Control: 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	12ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. Wash concentrate 20X: 1 bottle	25ml

Materials 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 450nm
5. Absorbance paper or paper towel

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and 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.

Reagent Preparation

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
 - The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
 - It is recommended that standards, control and serum samples be run in duplicate
 - Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities
1. Place the desired number of coated strips into the holder.
 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 μ l of TMB substrate and incubate for 10 minutes at room temperature. Add 100 μ l of stop solution.
8. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm

Calculation of Results

1. 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.
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 a Standard Curve

Calibrator mean OD = 0.8

Calibrator Factor (CF) = 0.5

Cut-off Value = $0.8 \times 0.5 = 0.400$

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. If 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 be greater than 1.2.

Interpretation

The following is intended as a guide to interpretation of Mumps IgG 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 Mumps IgG by ELISA
- $0.9-1.1$ Borderline positive. Follow-up testing is recommended if clinically indicated.
- >1.1 Detectable antibody to Mumps IgG by ELISA

References

1. Davidkin I; Valle M; Julkunen I. Persistence of anti-mumps virus antibodies after a two-dose MMR vaccination. A nine-year follow-up. *Vaccine* 1995;13(16):1617-22.
2. Chomel JJ; Robin Y; Durdilly R; Thouvenot D; Langlois M; Aymard M. Rapid direct diagnosis of mumps meningitis by ELISA capture technique. *J Virol Methods* 1997;68(1):97-104.
3. Nigro G; Nanni F; Midulla M. 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; Jongerius MC; van der Zwan CW; Plantinga AD; Kraaijeveld CA; Berbers GA. 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.

5. Novotný J. Properties and use of mumps viral antigen for detection of specific IgG and IgM antibodies in enzyme-linked immunosorbent assay. *Acta Virol* 1990;34(6):574-7.
6. Johnson CE; Kumar ML; Whitwell JK; Staehle BO; Rome LP; Dinakar C; Hurni W; Nalin DR. 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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