

Mumps IgG ELISA Kit

Prod. No.: DEIA363

Pkg. Size: 96T

INTENDED USE

The Mumps IgG Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Mumps virus in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service. This assay is intended for in-vitro diagnostic use only. Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

GENERAL DESCRIPTION

Mumps (Parotitis) is a common contagious disease with relatively moderate symptoms during childhood, but increasing complications, when adults are infected. The causative agent of mumps is a virus of the Paramyxoviridae family. The virus normally infects children at the age of 4 to 10. The infection is mainly transmitted by the airborne route, but is also spread by various objects contaminated by the patient saliva. The disease shows a seasonal prevalence with the greatest incidence in winter and spring. Both a mumps infection or vaccination lead to a persistent immunity. The typical symptom associated with a mumps infection is a „parotitis“ (swelling of the parotid glands). Additionally pathological involvement of the CNS and various glandular organs (pancreas, thymus, thyroids) is a typical feature of mumps. A mumps induced meningitis is one of the most frequent manifestations of the disease which can also appear without parotitis. The published data about the presence of meningitis vary between 1.4% and 66% of the clinically ill patients. In most cases there follows convalescence without complications. The recognition of Mumps in the laboratory is mostly done by detection of the infectious agent itself or by the determination of virus-specific antibodies. The serodiagnosis plays a major part. Besides the classical methods like complement fixation, hemagglutination and neutralisation tests have been introduced a series of modern assays like immunofluorescence, radioimmunoassay and enzyme immunoassay. The immunity of an individual is monitored by an IgG ELISA. Increasing IgG titers are helpful in the determination of the causative agent because cross reactions with Parainfluenza-Virus type 2 may lead to a wrong interpretation of the patient reports. In the early stage of the disease, significant IgM titers may be monitored.

PRINCIPLE OF THE TEST

The Mumps IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Mumps antigen is bound on

the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Mumps antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results. For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

REAGENTS PROVIDED

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Mikrotiter Strips: 12 strips with 8 breakable wells each, coated with a Mumps antigen (Strain Enders, ATCC VR-106). Ready-to-use.

Cut-Off Standard: 2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Mumps pneumoniae. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

Weak Positive Control: 2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Mumps. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

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Positive Control: 2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Mumps. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

Negative Control: 2 mL, protein solution diluted with PBS, contains no IgG antibodies against Mumps. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

Enzyme Conjugate: 15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

Substrate: 15 mL, TMB (tetramethylbenzidine). Ready-to-use.

Stop Solution: 15 mL, 0.5 M sulfuric acid. Ready-to-use.

Sample Diluent: 60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

Washing Buffer: 60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

Plastic Foils: 2 pieces to cover the microtiter strips during the incubation.

Plastic Bag: Resealable, for the dry storage of non-used strips.

MATERIALS REQUIRED BUT NOT PROVIDED

1. 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
2. Microtiter Plate Reader (450 nm)
3. Microtiter Plate Washer
4. Reagent tubes for the serum dilution
5. Bidistilled water

ASSAY PROCEDURE

1. Reagent And Sample Preparation

Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- 1) Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- 2) All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- 3) Standards and samples should be assayed in duplicates.
- 4) A standard curve should be established with each assay.
- 5) Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

2. Assay Steps

- 1) Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.

- 2) Pipet 100 µL each of the diluted (1:101) samples and the ready-to-use standards and controls respectively into the wells. Leave one well empty for the substrate blank.

- 3) Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.

- 4) Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

- 5) Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.

- 6) Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.

- 7) Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

- 8) Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.

- 9) Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).

- 10) To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.

- 11) After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

EVALUATION

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

1. Qualitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/-20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run. The positive control must show at least the double absorption compared with the cut-off standard.

2. Quantitative Evaluation

The ready-to-use standards and controls of the Mumps antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units

are printed on the labels of the vials. For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

ASSAY CHARACTERISTICS

Mumps ELISA	IgG	IgA	IgM
Intra-Assay-Precision	8.9 %	8.5 %	8.7 %
Inter-Assay-Precision	7.8 %	7.2 %	6.8 %
Inter-Lot-Precision	1.3 – 13.8 %	1.7 – 9.0 %	4.2 – 9.5 %
Analytical Sensitivity	1.28 U/mL	1.09 U/mL	1.23 U/mL
Recovery	72 – 101 %	80 – 129 %	89 – 104 %
Linearity	70 – 117 %	87 – 126 %	83 – 121 %
Clinical Specificity	100%	100%	100%
Clinical Sensitivity	100%	100%	100%

Cross-Reactivity: No cross-reactivity to Measles and Varicella zoster

Interferences: No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL

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