

ELISA kits available from ADI (see details at the web site)

Catalog#	ProdDescription
920-100-AIV	Chicken Anti-Avian Influenza virus (AIV) IgG ELISA kit
920-010-PAG	Swine/Pig Anti-Influenza A virus IgG ELISA kit
920-020-PAM	Swine/Pig Anti-Influenza A virus IgM ELISA kit
920-030-PAA	Swine/Pig Anti-Influenza A virus IgA ELISA kit
920-040-HAG	Human Anti-Influenza A virus IgG ELISA kit
920-050-HAM	Human Anti-Influenza A virus IgM ELISA kit
920-060-HAA	Human Anti-Influenza A virus IgA ELISA kit
920-110-AIM	Chicken Anti-Avian Influenza virus (AIV) IgM ELISA kit
600-640-PMY	Swine/Pig Myoglobin ELISA Kit
6250-40	Swine/Pig Haptoglobin ELISA kit
80186	Swine/Pig Serum Antibody detection ELISA kit, Qualitative
9000	Swine/Pig Albumin ELISA Kit, 96 tests, Quantitative
9020	Swine/Pig IgG (total) ELISA Kit, 96 tests, Quantitative
9080	Swine/Pig IgM ELISA Kit, 96 tests, Quantitative
920-110-AV	Chicken Anti-Anemia Virus (AV) Ig's ELISA kit
920-120-NDV	Chicken Anti-Newcastle Disease Virus (NDV) Ig's ELISA kit
920-130-IBV	Chicken Anti-Infectious Bronchitis Virus (IBV) Ig's ELISA kit
920-140-MDV	Chicken Anti-Marek's Disease Virus (MDV) Ig's ELISA kit
910-100-JEM	Mouse Anti-Japanese encephalitis virus (JEV) Ig's ELISA kit
910-110-JWM	Mouse Anti-Japanese encephalitis virus (JEV) Ig's WB kit, 12 tests
900-100-83T	Mouse Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
900-120-83T	Rabbit Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
900-140-83T	G. pig Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
900-150-83T	Monkey Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
900-160-83T	Human Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit

Instruction Manual No. M-520-100-HMG

Anti-Mumps Virus (parotitis) IgG

ELISA KIT Cat. # 520-100-HMG, 96 Tests

For Detecting Human IgG antibodies against Mumps Virus in Serum or Plasma

For In Vitro Research Use Only



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Kit Components (96 tests)	Cat #
Mumps IgG antigen coated strip plate, (8x12 strip or 96 wells) # 520101	1 plate
Mumps IgG Calibrator A, Negative Control (2 mL) #520102A, ready-to-use	1 vial
Mumps IgG Calibrator B Cut-off control (3 mL) #520102B, ready- to-use	1 vial
Mumps IgG Calibrator C Positive Control (2 mL) #520102C, ready-to-use	1 vial
controls contain 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane as preservative (or see lot sp. conc on the vial)	
Anti-Human IgG -HRP Conjugate, 20 ml #520103	1 bottle
Sample Diluent, 100 ml #520100SD	1 bottle
Wash buffer (20X) 50 ml # 520100WB	1 bottle
TMB Substrate Solution, 15 ml #520100TM	1 bottle
Stop Solution, 15 ml # 520100ST	1 bottle
Complete Instruction Manual, M-520-100-HMG	1

Intended Use

ADI's Mumps virus IgG ELISA kits are intended for the detection of IgG antibody to Mumps virus in human serum or plasma or normal or vaccinated individuals. This kit is for research use only, not for diagnostic or therapeutic use.

Introduction

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 disease shows a seasonal prevalence with the greatest incidence in winter and spring. Both a mumps infection or vaccination lead to a persistent immunity. Mumps is a contagious disease that is spread from person to person through contact with respiratory secretions such as saliva from an infected person. When an infected person coughs or sneezes, the droplets aerosolize and can enter the eyes, nose, or mouth of another person. Mumps can also be spread by sharing food and drinks. The virus can also survive on surfaces and then be spread after contact in a similar manner. A person infected with mumps is contagious from approximately 6 days before the onset of symptoms until about 9 days after symptoms start. The incubation period (time until symptoms begin) can be from 14–25 days but is more typically 16–18 days.

In developed countries, most children are immunized against measles by the age of 18 months, generally as part of a three-part **MMR vaccine (measles, mumps, and rubella)**. In endemic countries, WHO recommend that two doses of vaccine be given at six months and at nine months of age. **MMR II** vaccine (Merck) is a live virus vaccine for vaccination against measles (rubeola), mumps, and rubella (German measles). Attenuated Measles virus, derived from Enders' attenuated Edmonston strain and propagated in chick embryo cell culture, is used in MMR II vaccine. MMR II is a mixture of three live attenuated viruses, administered via injection.

Quality Control

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. The positive control must show at least double the OD of the cut-off standard. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

PERFORMANCE CHARACTERISTICS

Intra-Assay-Precision 4.32 %

Inter-Assay-Precision 9.27 %

Analytical Sensitivity 1.28 U/mL

Clinical Sensitivity 96.43 %

Clinical Specificity 99.49 %

Recovery 72-101 %

Linearity 82- 130 %

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.

Cross Reactivity

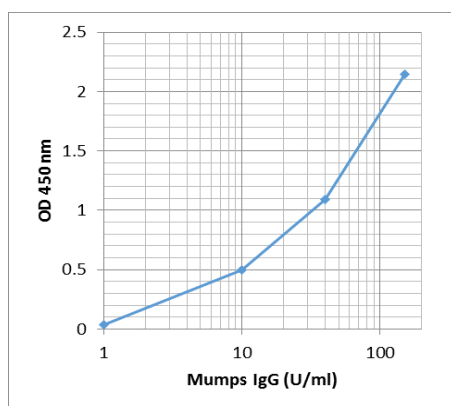
No cross reactivity to Measles and Varicella zoster.

References:

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	Results
A1, A2	Calibrator A Negative Control	0.009	
B1, B2	Calibrator B Cut-off standard	0.598	
C1, C2	Calibrator C Positive Control	1.672	
D1, D2	Sample 1		Negative
E1, E2	Sample 1		Positive

NOTE: These data are for **demonstration purpose only**. Use the values that are generated with each test.



4_ADI_ELISA

Calculation of Results

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log.

For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read from the standards curve.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in "Test Procedure" and re-assayed.

PRINCIPLE OF THE TEST

Alpha Diagnostic's mumps IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Mumps IgG antigens are 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 IgG takes place. After an incubation step, the plate is rinsed with wash solution, in order to remove unbound material. Bound antibodies are detected with anti-human-IgG peroxidase conjugate. After a further washing step, the substrate (TMB) solution is added. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The color is measured using an ELISA reader at 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color. Results are obtained by comparing the A450 of the samples with the supplied negative and positive calibrators.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. distilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

PRECAUTIONS

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. No reagents from different kit lots have to be used; they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), Diluted H₂SO₄ (1N, stop solution), and Thimerosal (0.02% v/v in conjugate diluent and HRP-conjugates).

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 1:100 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

REAGENTS PREPARATION

1. **Dilute Wash buffer 1:20 with water (eg. 10 ml washing buffer + 190 ml distilled water).** Store diluted buffer at 4°C for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.

All reagents must be at room temperature prior to their use.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots and should be stable for 3 months.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **All samples should be diluted 1:100 (5 ul samples in 500 ul sample diluent). Calibrators/controls are ready-to-use.** It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate.

1. Label or mark the microtiter well strips to be used on the plate. Dilute the wash buffer with water (1:20).
2. Dispense 100 ul diluent in 1 well to be used as blank. Pipet **100 ul of diluted samples** (dilute 1:100) & **ready-to-use calibrators/ controls** into appropriate wells in *duplicate*. See worksheet of a typical set-up on page 5. Cover the plate, mix gently for 5-seconds and **incubate at room temp for 60 min.**
3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 250-300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Add **100 ul anti-IgG-HRP conjugate** to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and **incubate for 30 minutes** at room temp (25-28°C).
5. **Wash the wells 3 times** as in step 3.
6. Add **100 ul TMB substrate solution.** Mix gently for 5-10 seconds. Cover the plate and **incubate for 20 minutes** at room temp. Blue color develops in positive controls and samples.
7. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (**blue color turns yellow**).
8. **Measure the absorbance at 450 nm** using an ELISA reader within 60 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Do not touch the bottom of the wells.

Interpretation of Results

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards.

Negative	<9 U/ml
Equivocal	9-11 U/ml
Positive	>11 U/ml

The results themselves should not be the only reason for any therapeutic consequences. They have to be correlated to other clinical observations and diagnostic tests.

Expected values

Ig Isotype	n	Interpretation		
		Positive	Equivocal	Negative
IgG	128	78.1 %	8.6 %	13.2 %