

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

Instruction Manual No. M-520-130-MMG

| Catalog# | ProdDescription |
|-------------------|--|
| 4200 | Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgG ELISA kit |
| 4205 | Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgM ELISA kit |
| 4220-AHB | Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) ELISA kit, Quantitative |
| 4300-AHG | Human Anti-Hepatitis A Virus IgG (HAV-IgG) ELISA kit, Quantitative |
| 4600 | Human Anti-Hepatitis C Virus (Anti-HCV) ELISA kit, Semi-Quantitative |
| 510-100-HRG | Human Anti-Rubella Virus IgG ELISA kit |
| 510-110-HRM | Human Anti-Rubella Virus IgM ELISA kit |
| 520-100-HMG | Human Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative |
| 520-110-HMM | Human Anti-Mumps Virus (parotitis) IgM ELISA, 96 tests, Quantitative |
| 520-120-HMA | Human Anti-Mumps Virus (parotitis) IgA ELISA, 96 tests, Quantitative |
| 520-200-HVG | Human Anti-Varicella Zoster Virus (chickenpox) IgG ELISA, 96 tests, Quantitative |
| 520-210-HVM | Human Anti-Varicella Zoster Virus (chickenpox) IgM ELISA, 96 tests, Quantitative |
| 520-220-HVG | Human Anti-Varicella Zoster Virus (chickenpox) IgA ELISA, 96 tests, Quantitative |
| 530-100-HMG | Human Anti-Measles IgG ELISA kit, 96 tests |
| 530-110-HMM | Human Anti-Measles IgM ELISA kit, 96 tests |
| 530-120-HMA | Human Anti-Measles IgA ELISA kit, 96 tests |
| 970-100-PHG | Human Anti-Polio Virus IgG ELISA kits, 96 tests, Quantitative |
| 540-110-DHM | Human Anti-Polio Virus IgM ELISA kits, 96 tests |
| 600-020-HRV | Human Anti-Rabies Virus IgG ELISA Kit, 96 tests, Quantitative |
| 600-120-HRV | Human Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA Kit, 2x 96 tests, |
| 600-220-HRV | Human Anti-Rabies Virus Nucleoprotein (RV-NP) IgG ELISA Kit, 2x 96 tests, |
| 600-300-100 | Human Anti-Meningococcal Group A Oligosaccharides-Diphtheria CRM197 IgG |
| 600-300-105 | Human Anti-Meningococcal Group CWY Oligosaccharides-Diphtheria CRM197 |
| 600-300-115 | Human Anti-Meningococcal Group ACWY Oligosaccharides-Diphtheria CRM197 |
| 600-370-CFP | Human Cardiac Fatty acid binding protein (FABP) ELISA kit |
| 600-410-CTN | Human Cardiac Troponin-I (Tn-I) ELISA Kit |
| 600-610-HMY | Human Myoglobin ELISA Kit |
| 700-140-KLM | Human Anti-KLH IgG (total) ELISA Kit, 2x 96 tests, Quantitative |
| 700-160-VAH | Human Anti-Vacmune/Immucotethel (KLH) IgG (total) ELISA Kit, 2x 96 tests, |
| 710-140-BSM | Human Anti-BSA IgG (total) ELISA Kit, 2x 96 tests, Quantitative |
| 80170 | Human Serum Antibody detection ELISA kit, Qualitative |
| 900-160-83T | Human Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit |
| 910-160-JEM | Human Anti-Japanese encephalitis virus (JEV) IgG specific ELISA kit |
| 910-170-JEM | Human Anti-Japanese encephalitis virus (JEV) IgM specific 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 |
| 920-400-HBG | Human Anti-Influenza B virus Ig's ELISA kit |
| 930-100-TTH | Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 940-100-DHG | Human Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 940-110-DHM | Human Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative |
| 940-200-DHG | Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit |
| 940-210-DHM | Human Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit |
| 950-100-AHA | Human Anti-Adenovirus IgA ELISA kit |
| 950-110-AHG | Human Anti-Adenovirus IgG ELISA kit |
| 950-120-AHM | Human Anti-Adenovirus IgM ELISA kit |
| 960-200-PHA | Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit, |
| 960-220-PHM | Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit, |
| 960-250-PHG | Human Anti-B. pertussis Pertactin IgG ELISA kit |
| 970-100-PHG | Human Anti-Poliomyelitis Virus 1-3 IgG ELISA Kit, 96 tests |
| 980-100-PHG | Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgG ELISA Kit, 96 |
| 980-110-PHM | Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgM ELISA Kit, 96 |
| 990-100-THA | Human Anti-Mycobacterium Tuberculosis IgA ELISA kit, 96 tests |
| 990-110-THG | Human Anti-Mycobacterium Tuberculosis IgG ELISA kit, 96 tests |
| 990-120-THM | Human Anti-Mycobacterium Tuberculosis IgM ELISA kit, 96 tests |
| AE-320420-1 tests | Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgG ELISA Kit, 96 |
| AE-320430-1 tests | Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgM ELISA Kit, 96 |
| AE-320520-1 | Human Anti-Zaire-Ebola virus IgG ELISA Kit, 96 tests |

Mouse Anti-Mumps IgG ELISA KIT

Cat. # 520-130-MMG, 96 Tests

For Detecting IgG antibodies against Mumps Virus
In Mouse Serum or Plasma

For Research use only (RUO)



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| Kit Components (96 tests) | qty |
|---|----------|
| Mumps antigen coated strip plate, (8x12 strip or 96 wells) # 520131 | 1 plate |
| Calibrator A, Negative Control, 2 mL #520-132A | 1 vial |
| Calibrator B, Cut-Off Control, 3 mL #520-132B | 1 vial |
| Calibrator C, Positive Control, 2 mL #520-132C | 1 vial |
| All calibrators contain 0.02 % methylisothiazolone as preservative | |
| Anti-Mouse IgG-HRP Conjugate, 20 ml, H-MsG.6BL (pink solution) | 1 bottle |
| Sample Diluent, 100 ml, #520-130-SD, (blue solution) | 1 bottle |
| Wash buffer (20X), 50 ml, #520-130-WB | 1 bottle |
| TMB Substrate Solution, 15 ml, #520-130-TB, (brown bottle) | 1 bottle |
| Stop Solution, 15 ml, #520-130-ST | 1 bottle |
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Intended Use

Mouse Anti-Mumps IgG ELISA Kit is an indirect ELISA suitable for quantifying IgG antibody activity specific for mumps in mouse serum, plasma or other qualified biological samples from vaccinated, immunized and/or infected mice.

This immunoassay is suitable for:

- Determining **immune status** relative to non-immune controls;
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and standardizing vaccine batches & protocols.

The assay is for research use only (RUO) and is not intended for therapeutic uses.

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 and 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

Interpretation of Results

The following data is derived from human samples.

| U | Interpretation |
|--------|----------------|
| < 9 | negative |
| 9 – 11 | equivocal |
| 10 | Cut-Off |
| > 11 | positive |

There is no information available for mouse samples. We recommend that the researchers establish basal levels for the control and vaccinated animals or establish their own know negative and positive controls.

Mouse Sample Testing

A panel of sera from non-vaccinated, adult mice (Balb/c, mixed sex) were tested in the assay at 1:100 dilution. A450 values ranged from 0.048 – 0.161.

We recommend that users establish sample dilutions that will give acceptable basal values for their animals. The actual test dilution can be varied from 1:50-1:500. Mice with no exposure to mumps should produce A450 values of <0.500 at a given dilution.

Quality Control

Blank values must be <0.100. Higher blank values are usually from inefficient washing. In case of high blank, re-run the standards and blanks until satisfactory values are established.

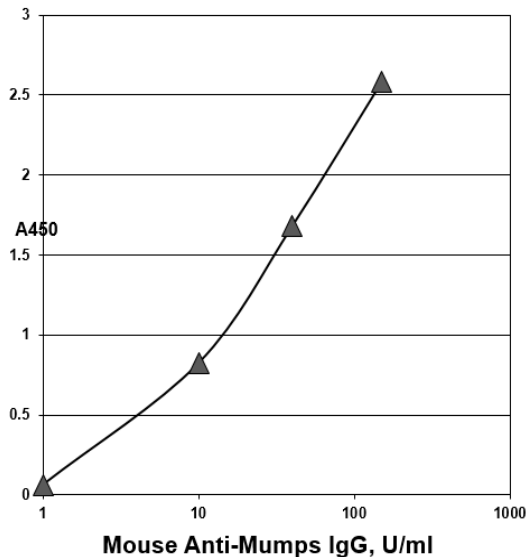
| | |
|------------------|---------------------|
| Substrate Blank | <0.100 |
| Negative Control | <0.200 and <Cut-Off |
| Cut-Off Control | 0.150 – 1.300 |
| Positive Control | >Cut-Off |

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, equipment, etc).

WORKSHEET OF A TYPICAL ASSAY

| Wells | Stds/samples | Mean A450 |
|--------|--------------|-----------|
| A1, A2 | Calibrator A | 0.009 |
| B1, B2 | Calibrator B | 0.598 |
| C1, C2 | Calibrator C | 1.672 |
| E1, E2 | Sample 1 | |
| F1, F2 | Sample 2 | |
| G1, G2 | Sample 3 | |
| H1, H2 | Sample 4 | |

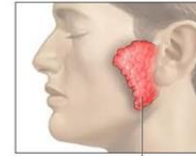
NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results. Actual values may differ from the above and may be lot specific. Lot specific and the values obtained for a given run should be used for calculation of sample values.



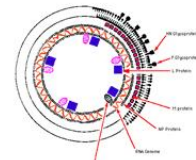
520-130-MMG-ELISA-Graph/ /nase1

CALCULATION OF RESULTS

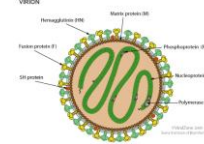
The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log. For the calculation of the standard curve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



Swollen parotid gland



the small hydrophobic (SH) protein.



Although there is only one serotype of the mumps virus and 12 genotypes (namely A, B, C, D, F, G, H, I, J, K, L, N) excluding (E and M). L-Zagreb vaccine strain comes under genotype N. There are several subgenotypes (D1, D2 G1, G2, H1, H2) which are designated by numerals after the letter. G2 is the current subgenotype circulating in the Indian subcontinent. Among vaccine strains, Jerryl Lynn and Rubini belong to genotype A, Urabe is genotype B. It is not clear yet if there is any clinical significance to the genotypes.

Mumps and Animal model- Mice are models for many human pathogens. However, mumps viruses are not known to replicate well or cause illness in adult mice. In most studies, mice were used to evaluate immune responses induced by different mouse cell-adapted or attenuated strains of mumps virus or to evaluate memory B cell responses after MMR vaccination. Newborn hamsters and mice injected with mumps viruses intracerebrally or intraperitoneally confirmed the neurotropism of the virus to both newborn hamster and mouse neurons, with differing neurovirulence among strains.

PRINCIPLE OF THE TEST

Alpha Diagnostic's Mumps IgG antibody test kit is based on the principle of the indirect ELISA. 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 60 minutes incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Anti-IgG peroxidase conjugate is added and incubated. After a further washing step, the substrate (TMB) solution is pipetted and incubated, 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 at 450 nm in an ELISA reader. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

MATERIALS AND EQUIPMENT REQUIRED

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

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

TMB (substrate), H₂SO₄ (stop solution)

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

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) will require dilution with sample diluent.

Mouse Sample Dilutions: An initial test dilution of 1:100 is recommended for most mice. Basal mumps antibody values may differ due to strains, age, sex, and potential or intentional exposure to mumps antigen. Therefore, final test dilution can be varied from 1:20-1:500. Vaccinated samples should be tested at higher dilutions or until sample values are within the range of the standards.

Sample Stability

Initial dilution of serum (1:10; or 5 ul serum into 45 ul diluent) into **Sample Diluent** is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for months stored frozen. Further test dilution (1:100 or more) should be performed within a week of the assay.

Example:

Initial (1:10): 5 ul serum + 45ul sample diluent (store below 10° C)

Further (1:100): 25 ul initial (1:10) + 225 ul sample diluent (use 100 ul x 2 for testing)

REAGENTS PREPARATION

Dilute Wash buffer(20x) 1:19 with distilled water (10 ml washing buffer + 190 ml distilled water). Store diluted buffer refrigerated for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes).

Bring all reagents to room temperature prior to 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). Prepare Wash Buffer prior to start of assay.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag, sealed with desiccant. **All samples should be diluted (see page 3). Calibrator provided are ready to use.**

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **100 ul of sample diluent (buffer blank), Calibrators and samples** (diluted) into appropriate wells in *duplicate*. See worksheet of a typical set-up on page 5. Mix gently by tapping and **incubate for 60 minutes at 37°C**.
3. Aspirate (or dump) the well contents; immediately, **wash the wells 3 times** with 250-300 ul of 1X wash buffer, then blot the plate on absorbent paper. An automated ELISA plate Washer may be used for best consistency; manual washing may be most convenient for fewer strips. Failure to wash the wells properly may lead to high blank and sample values.
4. Add **100 ul anti-Mouse IgG-HRP conjugate** to all wells. Mix gently by tapping and **incubate for 30 minutes** at room temperature (20 - 25° C).
5. **Wash the wells 3 times** as in step 3.
6. Add **100 ul TMB substrate solution**. Mix gently as above and **incubate for 15 minutes** at room temperature (20 - 25° C) in the dark. Blue color develops in positive controls and samples.
7. Stop the reaction by adding **100 ul of stop solution** to all wells. Tap gently for 5-10 seconds to have uniform color distribution (**blue color turns yellow**).
8. **Measure the absorbance at 450 nm** (using 620nm as reference is optional) using an ELISA reader within 30 minutes.

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 refrigerated in a sealed bag with desiccant. Do not touch the bottom of the wells.