

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

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
520-230-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/Immucoteth (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

Instruction Manual No. M-520-240-MVM

Mouse Varicella Zoster IgM ELISA KIT

Cat. # 520-240-MVM, 96 Tests

For the detection of mouse IgM antibodies against Varicella zoster in serum and plasma.

For In Vitro Research Use Only (RUO)



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Kit Components (96 tests)	
Varicella zoster antigen coated strip plate, (8x12 strip or 96 wells) # 520-241	1 plate
Calibrator A (Negative Control); 2.0 mL; #520-242A	1 vial
Calibrator B (Cut-off Control); 3.0 mL; #520-242B	1 vial
Calibrator C (Positive control); 2.0 mL; #520-242C	1 vial
Anti-Mouse IgM-HRP Conjugate, (20 ml) #520-243	1 bottle
Sample Diluent, 100 ml # 520-240-SD	1 bottle
Wash buffer (20X) 50 ml # 520-240-WB	1 bottle
TMB Substrate Solution, 15 ml #520-240-TM	1 bottle
Stop Solution, 15 ml # 520-240-ST	1 bottle
Complete Instruction Manual; M-520-240-MVM	1

Intended Use

ADI's Varicella Zoster IgM Antibody ELISA Test Kit has been designed for the detection of IgM class antibodies against Varicella Zoster in mouse serum and plasma (citrate, heparin). **This kit is for in vitro research use only.**

Introduction

Varicella-zoster virus (VZV) is known by many names, including chickenpox virus, varicella virus, zoster virus, and human herpes virus type 3 (HHV-3). VZV is closely related to the herpes simplex viruses (HSV), sharing much genome homology. It is one of eight herpes viruses known to infect humans and other vertebrates. It commonly causes chickenpox in children and adults and herpes zoster (shingles) in adults and rarely in children. As with the other herpes viruses, VZV causes both acute illness and lifelong latency. Before vaccination became widespread, acute primary infection (varicella or "chickenpox") was common during childhood--especially in temperate climates. Varicella usually is a benign and self-limiting illness but can be more severe in adults and in individuals with cellular immunodeficiency. These individuals are at much higher risk of pneumonia and disseminated disease with visceral involvement. Zoster typically presents as a painful, localized cutaneous eruption occurring along 1 or more contiguous dermatomes. Humans are the only known natural hosts of VZV. Transmission of VZV occurs through direct contact with infectious lesions or by inoculation of aerosolized infected droplets onto a susceptible mucosal surface.

The known envelope glycoproteins (gB, gC, gE, gH, gI, gK, gL) correspond with those in HSV; however, there is no equivalent of HSV gD. The most popular test detects VZV-specific IgM antibody in blood; this appears only during chickenpox or herpes zoster and not while the virus is dormant. **VZV vaccines:** Varivax (Merck) is a chickenpox vaccine for children, adolescents and adults. Zostavax is a vaccine for shingles for adults age 60 and older. Zostavax is a live vaccine developed by Merck & Co.

ADI's anti VZV IgM ELISA kit is an enzyme immunoassay for the quantitative determination of IgM class antibodies against Varicella Zoster Virus (chickenpox) in mouse serum or plasma. ADI also has separate ELISA kits to measure IgA and IgG classes.

There are no approved values for mouse samples so the recommended values for the positive samples are based upon human sample guidelines.

Sample Values:

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

In an in-house study apparently healthy research subjects showed the following results:

Ig isotype	n	Interpretations		
		positive	equivocal	negative
IgM	56	85.7%	3.6%	10.7%

Normal mouse samples:

Normal mouse samples (non-vaccinated samples) yielded values below the cut-off when tested at 1:100.

Precision

Intraassay: <12.49%
 Interassay: <10.02%

Specificity and species reactivity

This kit detects IgM subtype of mouse anti-VZV with no significant reactivity with the mouse IgA or IgG. This kit is not designed for other species. ADI has separate human anti-VZV Ig ELISA kits.

LIMITATIONS OF THE TEST:

This assay is intended for research use only – not for use in diagnostic procedures. 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 must 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 must be brought to room temperature (20 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. 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.

WORKSHEET OF A TYPICAL ASSAY

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results.

	OD
Calibrator A (Negative Control)	0.012
Calibrator B (Cut off Control)	0.479
Calibrator C (Positive Control)	1.147

CALCULATION OF RESULTS

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

The OD of the calibrators (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. The initial dilution of unknowns has been taken into consideration when reading the results from the graph. Results of unknowns of higher predilution must be adjusted for the dilution factor. Unknowns showing concentrations above the highest calibrator must be diluted as described in "Assay Procedure" and reassayed.

Run Validation Criteria

For an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank:** Absorbance value < 0.100
- **Negative Control:** Absorbance value < 0.200 and < Cut-off
- **Cut-off Control:** Absorbance value 0.150 – 1.300
- **Positive Control:** Absorbance value > Cut-off

If these criteria are not met, the test is not valid and must be repeated.

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control

$$0.42 = 0.86 / 2 = 0.43$$
$$\text{Cut-off} = 0.43$$

Results in Units [U]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = [\text{Units} = U]$$

Example: $1.591 \times 10 = 37 \text{ U (Units)}$

$$0.43$$

PRINCIPLE OF THE TEST

ADI's Varicella zoster IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Varicella zoster antigens are bound on the surface of the microtiter strips. Diluted unknowns are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Varicella zoster antigen takes place. After 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-mouse-IgM peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 15 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 IgM 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. Bidistilled 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 must 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 must be taken. Serum and reagent spills must be wiped off with a disinfecting solution (e.g., sodium hypochlorite, 5%) and must be disposed of properly. All reagents must 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 must be taken that the stoppers are not contaminated. Further a possible mix-up must 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 must be used, they should not be mixed among one another. All reagents must 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 must 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), 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 (Citrate, 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. Heat inactivation of samples is not recommended.

For the performance of the test the samples (not the standards) must be diluted 1:100 with ready-to-use sample diluent (e.g., 5 µL serum + 500 µL sample diluent and thoroughly mix with a vortex). **Do not dilute the calibrators.**

Vaccinated mouse samples

Although we recommend testing of mouse samples at 1:100, but other sample dilutions may be considered from 1:20 or higher to distinguish between the normal and vaccinated animals.

REAGENTS PREPARATION

1. **Dilute Wash buffer (20X)** 1:19 with distilled water. (e. g. **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. Dilute all samples 1:100 with the sample diluent. It is recommended to prepare a parallel replica plate containing all sample for quick transfer to the coated plate. **DO NOT dilute calibrators or controls. Dilute wash buffer stock (20X) 1:19 with distilled water.**

1. Label or mark the microtiter well strips to be used on the plate
2. Dispense **100 ul** diluent in 1 well to be used as blank. Pipet **100 ul of calibrators, controls, and diluted (1:100) samples** 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 37°C for 60 min.**
3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 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-mouse IgM-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 (20-25°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 15 minutes** at room temp (20-25 °C) in the dark (the incubation time may be varied by 5-10 min so as to get the maximum A450 of no greater than 2.5). **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** and 630nm as reference using an ELISA reader within 30 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.