

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
520-200-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-Vacumne/Immucothel (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	Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgG ELISA Kit, 96 tests
AE-320430-1	Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgM ELISA Kit, 96 tests
AE-320520-1	Human Anti-Zaire-Ebola virus IgG ELISA Kit, 96 tests

Instruction Manual No. M-520-260-BVG

Monkey Varicella Zoster Virus IgG (VZV-IgG) ELISA KIT

Cat. # 520-260-BVG, 96 Tests

For the quantitative detection of IgG antibodies against VZV
in monkey serum or plasma.



For In Vitro Research Use Only



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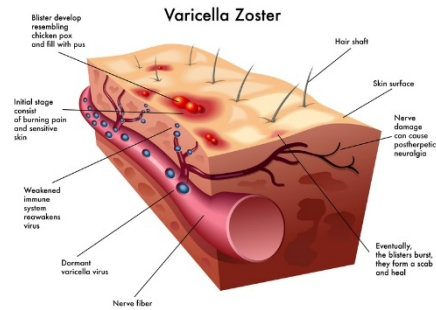
**DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE
KIT FOR ANY LOT SPECIFIC CHANGES.**

Kit Components (96 tests)	
Varicella zoster antigen coated strip plate, (8x12 strip or 96 wells) # 520261	1 plate
Anti-VZV IgG Calibrator A (Negative Control, 1 U/ml); 2 mL; #520262A	1 vial
Anti-VZV IgG Calibrator B (Cut-off standards, 10 U/ml); 2 mL; #520262B	1 vial
Anti-VZV IgG Calibrator C (weak positive control, 30 U/ml); 2 mL; #520262C	1 vial
Anti-VZV IgG Calibrator D (positive control, 150 U/ml); 2 mL; #520262D	1 vial
Anti-monkey IgG-HRP Conjugate, (15 ml) #520263	1 bottle
Sample Diluent, 60 ml # 520260-SD	1 bottle
Wash buffer (10X) 60 ml # 520260-WB	1 bottle
TMB Substrate Solution, 15 ml #520260-TM	1 bottle
Stop Solution, 15 ml # 520260-ST	1 bottle
Complete Instruction Manual; M-520-260-BVG	1

Intended Use

ADI's Varicella zoster IgG Antibody ELISA Test Kit has been designed for the detection of IgG class antibodies against Varicella zoster in monkey (rhesus, cynomolgous, and baboon) serum or plasma. This kit is for research use only (RUO) and not for diagnosis cure or prevention of the disease.

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 chicken-pox 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.

Normal Values (human)

These values are derived from human samples to be used as general guidance as there is no monkey specific data available.

Negative	Equivocal	Positive
<8	8-12	>12 U/ml

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

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

Monkey Sample

Serum samples from a mixed population of adult monkeys (rhesus, cynomolgous, and baboon) were tested at 1:100 sample dilution. Given the low basal values in most monkeys, it is possible to test the samples at 1:50 dilution to improve sensitivity.

Sample	Baboon	Rhesus	Cynomolgus
1	0.430	0.021	0.160
2	0.028	0.178	0.014
3	0.018	0.016	0.014
4	0.019	0.016	0.012
5	0.058	0.041	0.013
6	0.042	0.297	0.170
7	0.142		
8	0.273		

The values are absorbance at 450nm.

Antigen Specificity and Species reactivity

VZV (Ellen) is grown in human Fibroblast cells is extracted lysed cells using proprietary methods. The antigen preparation is partially purified to reduce host cell components and contains predominantly VZV antigens. The antigen is inactivated using detergents. However, it must be treated as if infectious and properly disposed. The kit will detect antibodies to several antigenic proteins of VZV.

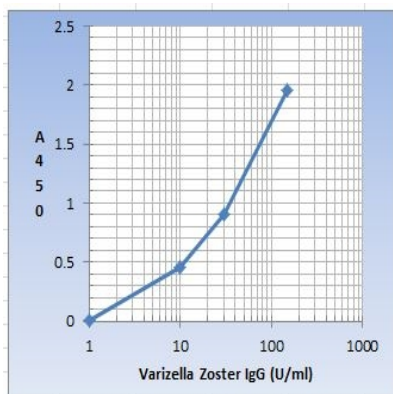
This kit is designed to be used in monkey samples and it has been tested with rhesus, cynomolgous and baboon samples. The anti-monkey IgG or anti-human IgG antibodies have significant cross reactivity. However, we recommend using human VZV kit for human samples for better sensitivity. ADI has similar kits for mouse samples for testing the efficacy of vaccines in mice and other animals.

References: Felsenfeld AD (1979) J. Gen. Virol. 42, 171-178; Soike KF (1987) Med Virol. 22, 307-313; Myers G (1992) J. Infec. Dis. 166, !S48-50; Soike KF (1984) Adv. Vet. Sci. Com. Med. 28, 151-199; Gray WL (2004) Rev. Med. Virol. 14, 363-381; Felsenfeld AD (1977) Infect. Immun.. 15, 807-812; Gray WL (1995) J. Med. Primatol. 24, 246-251

WORKSHEET OF A TYPICAL ASSAY

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

Serum	U/ml	Net A450
Calibrator A (Negative Control)	1	0.032
Calibrator B (Cut off std)	10	0.499
Calibrator C (weak positive Control)	30	1.011
Calibrator D (Positive Control)	150	2.147

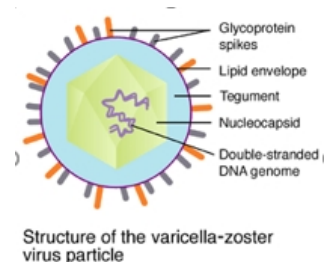


Kits-spec-XL
Typical Std Curve (do not use this for sample calculation)

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 have to be adjusted for the dilution factor. Unknowns showing concentrations above the highest calibrator have to be diluted as described in "Assay Procedure" and reassayed.



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.

Varicella Virus Infection in monkey

Human VZV is specific for human host. Simian varicella virus (SVV), the etiologic agent of naturally occurring varicella in primates, is genetically and antigenically closely related to human varicella zoster virus (VZV). Experimental inoculation of mice, rats and nonhuman primates (NHP) with VZV results in seroconversion but not varicella. There is a scarcity of the human, mouse (animal) and simian antibody ELISA kits so we do not know the extent of antibody cross reactivity between human and simian VZV antigens.

PRINCIPLE OF THE TEST

ADI's Varicella zoster IgG antibody test kit is based on the principle of the indirect ELISA. Varicella zoster antigen is bound on the surface of the microtiter strips. Diluted unknowns are pipetted into the wells of the microtiter plate. A binding between the IgG 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 Antibody-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.

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! All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. 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. 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.

REAGENTS PREPARATION:

1. **Dilute Wash buffer 1:10 with water. (Dilute 60 ml stock with 940 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.

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), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

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) have to be **diluted 1:50** with ready-to-use sample diluent (e.g. 5 µL serum + 250 µL sample diluent). Run in test in duplicate (100 ul/well). Do not dilute the calibrators.

REAGENTS PREPARATION

2. **Dilute Wash buffer** 1:10 with water. (**Dilute 60 ml stock with 940 ml distilled water**) Store diluted buffer at 4oC 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 6 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 1:50 with the sample diluent. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. **DO NOT dilute calibrators or controls. Dilute wash buffer stock (10X) 1:10 with distilled water.**

1. Label or mark the microtiter well strips to be used on the plate
- 2.
3. Dispense **100 ul** diluent in 1 well to be used as blank. Pipet **100 ul of calibrators, controls, and diluted 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 room temp (25-28oC) for 60 min.**
4. 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.
5. Add **100 ul anti-monkey 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 (18-26oC).
6. **Wash the wells 3 times** as in step 3.
7. 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.
8. 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**).
9. **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.