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
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 (chikenpox) IgG ELISA, 96 tests, Quantitative
520-210-HVM	Human Anti-Varicella Zoster Virus (chikenpox) IgM ELISA, 96 tests, Quantitative
520-220-HVG	Human Anti-Varicella Zoster Virus (chikenpox) 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-Poliomyelitis Viruses 1-3 IgG ELISA Kit, 96 tests
970-120-PMG	Mouse Anti-Poliomyelitis Virus 1-3 IgG ELISA Kit, 96 tests
970-130-PRG	Rabbit Anti-Poliomyelitis Virus 1-3 IgG ELISA Kit, 96 tests
970-130-PRM	Rabbit Anti-Poliomyelitis Virus 1-3 IgM ELISA Kit, 96 tests
970-150-PMG	Monkey Anti-Poliomyelitis Virus 1-3 IgG ELISA Kit, 96 tests
970-160-VPG	Mouse Anti-Poliomyelitis Virus 1 Viral Protein 1 (Sabin; POLV1-VP1) IgG ELISA
970-165-VPG	Rabbit Anti-Poliomyelitis Virus 1 Viral Protein 1 (Sabin; POLV1-VP1) IgG ELISA
970-170-VPG	Human Anti-Poliomyelitis Virus 1 Viral Protein 1 (Sabin; POLV1-VP1) IgG ELISA
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
700-140-KLM	Human Anti-KLH IgG (total) ELISA Kit, 2x 96 tests, Quantitative
700-160-VAH	Human Anti-Vacmune/Immucothel (KLH) IgG (total) ELISA Kit,2x 96 tests,
710-140-BSM	Human Anti-BSA IgG (total) ELISA Kit, 2x 96 tests, Quantitative
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
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-970-180-PRG

Rat Anti-Poliomyelitis Virus 1-3 IgG ELISA KIT Cat. # 970-180-PRG

For the detection of IgG antibody to Polio viruses 1-3 in Rat serum or plasma.

For In Vitro Research Use Only

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Kit Components (96 tests)	
Polio viruses antigens 1-3 antigen coated strip plate, (8x12 strip or 96 wells) # 970-181	1 plate
Std A (0.65 mL; Rat Anti-Polio virus 1-3 IgG 6.25 U/ml #970182A	1 vial
Std B (0.65 mL; Rat Anti-Polio virus 1-3 IgG 12.5 U /ml #970182B	1 vial
Std C (0.65 mL; Rat Anti-Polio virus 1-3 IgG 25.0 U /ml #970182C	1 vial
Std D (0.65 mL; Rat Anti-Polio virus 1-3 IgG 50 U /ml #970182D	1 vial
Std E (0.65 mL; Rat Anti-Polio virus 1-3 IgG 100 U /ml #970182E	1 vial
All standards are calibrated to an internal reference using arbitrary units.	
Rat Anti-Polio Vaccine IgG positive control (0.65 ml) #970183 See lot specific values on the vials	1 vial
Anti-Rat IgG-HRP Conjugate,100X (0.15 ml) #H-RsG-112b	1 bottle
Sample/Conjugate Diluent (20X), 10ml # SD-20T	1 bottle
Low NSB Sample Diluent (green color), 30 ml # TBTm	1 bottle
Wash buffer (10X) 60 ml # 970180-WB	1 bottle
TMB Substrate Solution, 12 ml #80091	1 bottle
Stop Solution, 12 ml # 80101	1 bottle
Complete Instruction Manual #970-180-PRG	1

Intended Use

ADI Polio viruses 1-3 IgG ELISA Kit is intended for the detection of IgG antibody to Polio virus in Rat serum or plasma of vaccinated mice. This kit is for in vitro research use only (RUO), not for therapeutic use.

Introduction

Poliomyelitis, often called polio or infantile paralysis, is an acute viral infectious disease spread from person to person, primarily via the fecal-oral route. Different types of paralysis may occur, depending on the nerves involved. The term poliomyelitis is used to identify the disease caused by any of the three serotypes of poliovirus. Type 1 (Brunhilde): often with severe symptoms Type 2 (Lansing): with milder symptoms Type 3 (Leon): rare, but with severe symptoms. The virus enters the central nervous system in about 3% of infections. Most patients with CNS involvement develop non-paralytic aseptic meningitis, with symptoms of headache, neck, back, abdominal and extremity pain, fever, vomiting, lethargy and irritability. A laboratory diagnosis is usually made based on recovery of poliovirus from a stool sample or a swab of the pharynx. Antibodies to poliovirus can be diagnostic, and are generally detected in the blood of infected patients early in the course of infection. Two types of **vaccines** are used throughout the world to combat polio. The first is Salk vaccine, or inactivated poliovirus vaccine (**IPV**), is based on three wild, virulent reference strains, Mahoney (type 1), MEF-1 (type 2), and Saukett (type 3) polio viruses, grown in a type of monkey kidney tissue culture (Vero cell line), which are then inactivated with formalin. Oral polio vaccine (**OPV** or Sabin's vaccine) is a live-attenuated vaccine, produced by the passage of the virus through non-human cells at a sub-physiological temperature, which produces spontaneous mutations in the viral genome. This vaccine is unable to replicate in the brain.
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QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The A450. of the blanks should be <0.50 and the highest standard >1.00.
2. Rat anti-Polio virus IgG control should be within the range specified on the vial.
3. High blank values >0.300 are usually due to inefficient washing, particularly after the antibody-HRP conjugate step. Increase the # of washing and make sure to tap the plates over a paper towels after the final wash or in between washes if washing manually.

Rat Sample Testing

The following is intended as a guide to interpretation of Polio virus IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

A testing of 5 non-vaccinate adult Rat (Sprague-Dawley) at 1:100 in Low nsb diluent produced A450 values in the range of 0.200-0.300. It is important to establish the antibody levels in a pool of mice that are non-vaccinated and not compare it with the standards or zero values. All vaccinated animals must have A450 at least 2X the values of the non-vaccinated to be considered positive.

Polio Vaccine Testing in Animals & Humans

Human samples obtained from vaccinated individuals tested positive using the polio virus antigen coated used in the Rat kit. Rabbits and mice vaccinated with Ipol vaccine also produced high tittered antibodies.

ADI also cloned and expressed Polio virus 1 (Sabin) viral protein 1 (Polv1-VP1, Cat# POLV15-R-10) and vaccinated mouse and rabbits. The resulting antiserum reacted with the human polio viruses 1-3 antigens used in this test. Similarly, rabbit anti-recombinant Polio VP1 protein antiserum (#POLV15-S) also reacted with the polioviruses 1-3 antigens used in the Rat anti-polio virus ELISA.

Specificity

Polio viruses' 1-3 antigens are used in the kit. Therefore, antibodies to all three polio serotype will be detected in this test. Anti-Rat IgG-HRP conjugate has been optimized to detect all IgG subtypes but not the IgM or IgA. It is possible to use anti-IgM specific conjugate to detect Rat IgM polio antibodies.

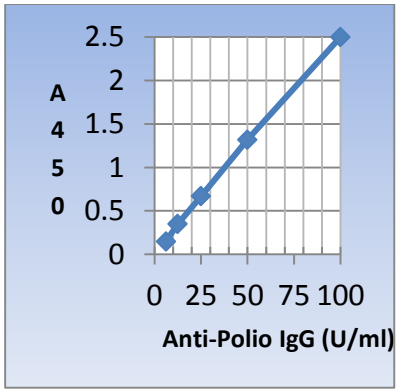
References

Pinheiro FP et al; World Health Stat Q 50(3/4):161-169, 1997; Gubler DJ et al; Infect Agents Dis 2:383-393, 1993; Wu SJ et al Clin Diagn Lab Immunol1997; 4(4):452-7; Lam SK; Clin Diagn Virol 1998;10(1):75-8; Rossi CA; 1995-1996. Am J Trop Med Hyg 1998;59(2):275-8 2008-12-18

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	A450	Net A450 (minus blank)
Sample Diluent or Blanks	0.00	0.100	
Rat Anti-Polio Virus IgG Std A	6.25	0.250	0.15
Rat Anti-Polio Virus IgG Std B	12.5	0.435	0.335
Rat Anti-Polio Virus IgG Std C)	25	0.87	0.77
Rat Anti-Polio Virus IgG Std D	50	1.43	1.33
Rat Anti-Polio Virus IgG Std E	100	2.60	2.50



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,) either on lin-lin graph paper or point-to-point curve fit. The initial dilution of unknowns (1:200) has been taken into consideration when reading the results from the graph. Therefore, do not multiply the sample values if used at 1:200 dilution. Results of unknowns of higher predilution (e.g., 1:400 have to be adjusted for the dilution factor and multiplied by 2).

Unknowns showing concentrations above the highest calibrator have to be diluted as described in “Assay Procedure” and re-assayed.

IPOL®, Poliovirus Vaccine Inactivated, produced by Sanofi, contains three types of polioviruses: Type 1 (Mahoney), Type 2 (MEF-1), and Type 3 (Saukett). IPOL vaccine is replicated in Vero cells. Each dose (0.5 mL) of trivalent vaccine is formulated to contain 40 D antigen units of Type 1, 8 D antigen units of Type 2, and 32 D antigen units of Type 3 poliovirus. Poliovirus Vaccine induces the production of neutralizing antibodies against each type of virus which are related to protective efficacy. There are 57 nucleotide substitutions which distinguish the attenuated Sabin 1 strain from its virulent parent (the Mahoney serotype), two nucleotide substitutions attenuates the Sabin 2 strain, and 10 substitutions are involved in attenuating the Sabin 3 strain. The primary attenuating factor common to all three Sabin vaccines is a mutation located in the virus's internal ribosome entry site (IRES) which alters stem-loop structures, and reduces the ability of poliovirus to translate its RNA template within the host cell. The attenuated poliovirus in the Sabin vaccine replicates very efficiently in the gut, the primary site of infection and replication, but is unable to replicate efficiently within nervous system tissue.

ADI’s Rat anti-polio virus IgG ELISA kit detects antibodies to the three subtypes of polio viruses that are currently used in vaccines. Rat Anti-Polio Virus IgG from mice vaccinated with the IPOL vaccine are included in the kit as positive control.

PRINCIPLE OF THE TEST

ADI’s Polio virus IgG ELISA Kit is based on the principle of the enzyme immunoassay (EIA or ELISA). Diluted serum or plasma samples are added to wells coated with purified Polio virus antigens. Polio virus IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme that produced blue color. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. 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! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. 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. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. 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), 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 1:200 with 1X-sample diluent (e.g. 5 µL serum + 195 µL sample diluent). Do not dilute the calibrators.

We recommend making an initial stock of 1:10 in 1X-sample diluent. The antibodies are stable in this buffer and kept at 4oC for up to 2-4 weeks. Make additional test dilution of samples from 1:10 stock (e.g., for 1:200 dilution, dilute 1:10 stock by another 1:20 or 15-ul of 1:10 stock and 285 ul of Low Nsb diluent .

We recommend 1:200 dilution of Rat sample in Low NSb diluent (green). This buffer keeps the non-specific binding and the final A450 <0.300. If samples are diluted 1:200 in 1X sample/conjugate diluent then the non-specifina A450 of naïve Rat sample may be higher (0.5-0.8).

REAGENTS PREPARATION

1. **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.

2. **Prepare 1X Sample/Conjugate Diluent**. Prepare 1X working stock by dilution1:20 with water. Dilute 1 ml stock in 19 ml water. This diluent can be used to prepare initial sample stock of 1:10 (or 1:200 etc) and dilute the antibody-HRP conjugate.

3. **Prepare 1X anti-Rat IgG-HRP conjugate**. Stock is provided as 100X stock. Dilute 10 ul stock conjugate in 1 ml of 1X sample diluent prepare above. Prepare 12 ml working stock for each strip or 12 ml for full plate assay (120 ul stock conjugate in 12 ml of 1X conjugate diluent)..

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^{O}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^{O}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 1:200 with the sample diluent (see page 3 for recommended preparation of the sample dilution). 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. Dispense **100 ul** 1X sample diluent in duplicate wells to be used as blank. Pipet **100 ul of** calibrators, controls, and diluted samples into appropriate wells in *duplicate*. Cover the plate, mix gently for 5-seconds and incubate at room temp (25-28oC) 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-Rat IgG-HRP conjugate** to all well. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp (18-26oC).

5. Wash the wells 4 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. **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^{O}C. Do not touch the bottom of the wells.