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

Catalog#	ProdDescription
970-100-PHG	Human Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests,
970-120-PMG	Mouse Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests,
5720-100-AH1	Mouse Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests,
970-140-PRM	Mouse Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgM ELISA Kit, 96 tests,
970-150-PMG	Monkey Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests,
970-160-VPG	Mouse Anti-Poliomyelitis VP 1 (Sabin; POLV1-VP1) IgG ELISA Kit,
970-165-VPG	Mouse Anti-Poliomyelitis VP1 1 (Sabin; POLV1-VP1) IgG ELISA Kit,
970-170-VPG	Human Anti-Poliomyelitis VP1 1 (Sabin; POLV1-VP1) IgG ELISA Kit
5720-100-AH1	Rat Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests,
POLV11-S	Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV vaccine: Mahoney, MEF-1, and Saukett)
antiserum	
POLV12-M	Mouse monoclonal Anti-Poliomyelitis Virus 1-3 IgG, aff pure
POLV13-A	Anti-Poliomyelitis Virus 1-3 IgG
POLV13-BTN	Anti-Poliomyelitis Virus 1-3 IgG-Biotin Conjugate
POLV13-FITC	Anti-Poliomyelitis Virus 1-3 IgG-FITC Conjugate
POLV13-HRP	Anti-Poliomyelitis Virus 1-3 IgG-HRP Conjugate
POLV14-M	Mouse monoclonal Anti-Poliomyelitis Virus 1 IgG, aff pure
POLV15-C	Recombinant Poliomyelitis VP 1 (Sabin; POLV1-VP1) control for Western blot
POLV15-R-10	Recombinant (E. Coli) Poliomyelitis VP 1 (Sabin; POLV1-VP1, 302-aa; full
length,	
POLV15-S	Anti-Poliomyelitis Virus 1 Viral Protein 1 (Sabin; POLV1-VP1) antiserum
POLV16-S	Anti-Poliomyelitis Virus 1 (LSc,2ab strain) antiserum, neutralizing
POLV21-M	Mouse monoclonal Anti-Poliomyelitis Virus 2 IgG, aff pure
POLV22-S	Anti-Poliomyelitis Virus 2 (P712,Ch,2ab strain) antiserum, neutralizing
POLV23-S	Anti-Poliomyelitis Virus 2 (sabin strain, native) antiserum, neutralizing
POLV31-M	Mouse monoclonal Anti-Poliomyelitis Virus 3 IgG, aff pure
POLV32-S	Anti-Poliomyelitis Virus 3 (Leon1,Ch,2ab strain) antiserum, neutralizing
POLV33-S	Anti-Poliomyelitis Virus 3 (sabin strain, native) antiserum, neutralizing
4200	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgG ELISA kit
4205	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgM ELISA kit
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
520-200-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
530-100-HMG	Human Anti-Measles IgG ELISA kit, 96 tests
530-110-HMM	Human Anti-Measles IgM ELISA kit, 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
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
930-100-TTH	Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-200-DHG	Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit
950-110-AHG	Human Anti-Adenovirus IgG ELISA kit
950-120-AHM	Human Anti-Adenovirus IgM 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

Instruction Manual No. M-5720-100-AH1

Mouse Anti-AH1 peptide (gp70 H2-Ld-restricted epitope) IgG ELISA KIT

Cat. # 5720-100-AH1; 96 Tests

For the detection of IgG antibody to AH1 IgG in Mouse serum or plasma or other biological fluids

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

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Your Molecular & Cell Technology Partner

Mouse Anti-AH1 peptide IgG ELISA KIT Cat. # 5720-100-AH1 (96 tests)

Kit Components (96 tests)	
AH1 peptide antigen coated strip plate, (8x12 strip or 96 wells) # 5720-101	1 plate
Anti-AH1 peptide IgG Std A (1 mL; 3 U/ml) #5720102A	1 vial
Anti- AH1 peptide IgG Std B (1 mL; 10 U/ml) #5720102B	1 vial
Anti- AH1 peptide IgG Std C (1 mL; 30 U/ml) #5720102C	1 vial
Anti- AH1 peptide IgG Std D (1 mL; 90 U/ml) #5720102D	1 vial
Anti-Mouse IgG-HRP **Conjugate**, (0.15 ml, 100X) #5720103	1 vial
Sample Diluent (20X), 10 ml # SD-20T	1 bottle
Wash buffer (100X) 10 ml # WB-100	1 bottle
TMB Substrate Solution, 12 ml # 80091	1 bottle
Stop Solution, 12 ml # 80101	1 bottle
Complete Instruction Manual	1

Intended Use

ADI **AH1 peptide IgG** ELISA Kit is intended for the detection of IgG antibody to AH1 peptide in AH1-vaccinated Mouse serum, plasma or other biological fluids. The kit is well suited for testing human polio vaccine formulations in Mouse. The kit contains no active virus or bacteria. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

Introduction

Vaccines containing tumor antigens or mimotope of tumor antigens, are being used in cancer immunotherapy with the intention of eliciting an increase in the number of T cells that cross-react with the native tumor antigen. A variety of mimotope have been identified from a mouse colon tumor that elicits a range of tumor protection following vaccination. Using the mouse colon carcinoma CT26, a peptide mimic of the immunodominant self-antigen gp70422-431 (AH1; SPSYVYHQF, mol wt 1127.2). This AH1 sequence (amino acids 6 to 14) is the H2-Ld-restricted epitope derived from gp-70 (endogenous retroviral gene product envelope glycoprotein 70), which is expressed in CT26 (colon carcinoma cells) and numerous other tumor cell lines. CD8+ T cell responses against the AH1 epitope, GP70423–431, protect against tumor challenge with the CT26 tumor cell line. Expression of AH1 in normal tissues induces tolerance in the T cell repertoire. Subsequently, vaccination with the AH1 epitope alone is poorly immunogenic (5, 7). Vaccines using variants of the AH1 epitope, however, induce robust AH1-reactive responses that protect prophylactically and therapeutically against CT26 tumor challenge.

Mouse anti-AH1 peptide IgG ELISA kit is designed to assess the presence of AH1 antibodies in vaccinated or naïve animals.

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the blanks should be < 0.200.
2. The A450 value of the highest std (90 U/ml should be >1.00.

Mouse sample testing

A population of non-vaccinated, healthy adult mice (Balb/C) were tested for the basal level of the antibodies to polio antigens by ELISA at a serum dilution of 1:100. Most samples showed A450 values of <0.200. This should be considered base value or background values. Animals showing significantly higher values should be considered positive or an induction of antibodies due to vaccination. Users must establish their reference values for their strains, sex, age, and exposure to the polio virus or vaccines.

Samples	Blank	R1	R2	R3	R4	R5	R6	R7	R8
A450	0.090	0.100	0.019	0.98	0.009	0.15	0.16	1.56	0.11

INTERPRETATION

There are no recommended guidelines for AH1 antibodies in Mouse. Each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations, exposure to the virus or vaccination.

Specificity

Highly purified AH1 peptide is used in the kit. Therefore, antibodies to AH1 will be detected in this test. Anti-Mouse IgG-HRP conjugate has been optimized to detect all IgG but not the IgM or IgA.

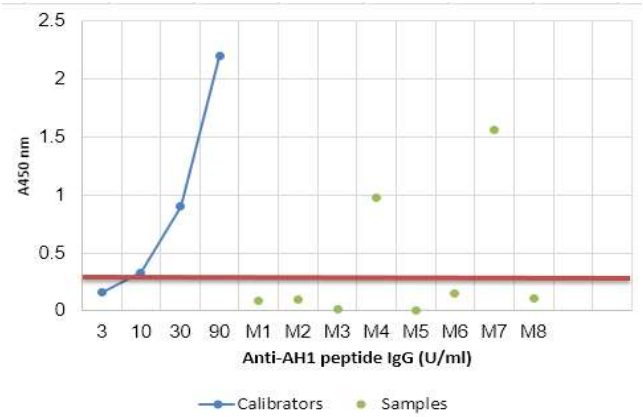
This kit is designed to mouse sample only. ADI can custom design a kit for other animals or human studies.

References:

Kemmler CB (2011) J. Immunol. doi:10.4049/jimmunol.1101555; DeLeo AB (1977) J. Exp. Med. 146, 720-734; Huuang A Y (1996) PNAS 93, 9730-9735; Slansky JE (2000) Immunity 13, 529-538; McMahan RH (2006) J. Clin. Invest. 116, 2543-2551

WORKSHEET OF A TYPICAL ASSAY

Stds	U/ml	Average A450	Net A450
Sample diluent (blank)	0	0.1	-
Mouse Anti-AH1 IgG	3	0.26	0.16
Mouse Anti-AH1 IgG	10	0.43	0.33
Mouse Anti-AH1 IgG	30	1.0	0.9
Mouse Anti-AH1 IgG	90	2.302	2.2
Mouse Sample 1		0.28	0.18



*/2-Nas/5720-100-AH1-ELISA-graph

Typical Std Curve (do not use this for sample calculation)

Red line represents an arbitrary cut-off of A450 =0.30A. Mouse sample when tested at 1:100 and showing A450 values higher than the cut-off may be considered positive for AH1 IgG.

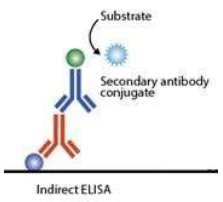
CALCULATION OF RESULTS

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 (1:100) has been taken into consideration when reading the results from the graph. Therefore, do not multiply the sample values if used at 1:100 dilutions. Results of unknowns of higher predilution (e.g., 1:500 have to be adjusted for the dilution factor and multiplied by 5). Unknowns showing concentrations above the highest std have to be diluted as described in "Test Procedure" and reassayed.

All samples above the cut-of values may be considered positive for the presence of anti-AH1 IgG.

PRINCIPLE OF THE TEST



ADI's AH1 IgG ELISA Kit is based on the principle of the enzyme immunoassay (EIA). Diluted patient serum is added to wells coated with purified AH1 antigen. AH1 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. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. 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), 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.

Mouse samples (not the standards) have to be diluted 1:100 with ready-to-use sample diluent (e.g. 5 µL serum + 495 µL sample diluent). Prepare at least 250-300 ul of diluted samples for testing. Do not store diluted samples beyond the assay date.

REAGENTS PREPARATION

1. **Dilute Wash** buffer 1:100 with water. (**Dilute 10 ml stock with 990 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. **Sample Diluent (Dilute 1:20 with water** (1 ml stock and 19 ml distilled water). Use 1x diluent for the dilution of the sample and to dilute the antibody-HRP Conjugate. Store at 2-4oC.

3. **Antibody-HRP Conjugate-**Stock is provided as 100x. **Dilute 1:100** with 1X sample diluent (10 ul stock in 990 ul 1x sample diluent). Prepare 1 ml for each strip or 10 ml for full plate. Store at 2-4oC until used. Do not store diluted antibody conjugate beyond the assay date.

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 1:100 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.

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 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.

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 antibody-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).

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 **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^{o}C. Do not touch the bottom of the wells.