

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

950-100-AHA Human Anti-Adenovirus IgA ELISA kit, 96 tests, Quantitative  
950-110-AHG Human Anti-Adenovirus IgG ELISA kit, 96 tests, Quantitative  
950-120-AHM Human Anti-Adenovirus IgM ELISA kit, 96 tests, Quantitative  
510-215-HEA Human Anti-Epstein Barr Virus (EA) IgA ELISA Kit, 96 tests  
510-220-HEG Human Anti-Epstein Barr Virus (EA) IgG ELISA Kit, 96 tests  
510-225-HEM Human Anti-Epstein Barr Virus (EA) IgM ELISA Kit, 96 tests  
510-200-HEA Human Anti-Epstein Barr Virus (EBNA-1) IgA ELISA Kit, 96 tests  
510-205-HEG Human Anti-Epstein Barr Virus (EBNA-1) IgG ELISA Kit, 96 tests  
510-210-HEM Human Anti-Epstein Barr Virus (EBNA-1) IgM ELISA Kit, 96 tests  
510-230-HEA Human Anti-Epstein Barr Virus (VCA) IgA ELISA Kit, 96 tests  
510-235-HEG Human Anti-Epstein Barr Virus (VCA) IgG ELISA Kit, 96 tests  
510-240-HEM Human Anti-Epstein Barr Virus (VCA) IgM ELISA Kit, 96 tests

960-110-PHG Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG, 96 tests,  
960-120-PHG Mouse Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG ELISA kit,  
960-130-PMG Mouse Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative  
960-140-PMM Mouse Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative  
960-150-PRG Rabbit Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative  
960-160-PRM Rabbit Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative  
960-170-PMG G. pig Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative  
960-180-PMM G. pig Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative  
960-200-PHA Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit,  
960-205-PHA Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit,  
960-210-PHG Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG ELISA kit,  
960-220-PHM Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit,  
960-225-PHM Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit,  
960-230-PGG Mouse Anti-B. pertussisPertactin IgG ELISA kit, 96 tests  
960-240-PRG Rabbit Anti-B. pertussisPertactin IgG ELISA kit, 96 tests  
960-250-PHG Human Anti-B. pertussisPertactin IgG ELISA kit, 96 tests  
960-260-PMG Monkey Anti-B. pertussisPertactin IgG ELISA kit, 96 tests  
960-300-FMG Mouse Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96  
960-310-FMM Mouse Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96  
960-320-FRG Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96  
960-330-FRM Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96  
960-340-FHG Human Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96  
960-350-FHM Human Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96

940-100-DHG Human Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
940-120-DMG Mouse Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
940-125-DMM Mouse Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative  
940-130-DRG Rabbit Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
940-135-DRM Rabbit Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative  
940-140-DGG Guinea Pig Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
940-145-DGM Guinea Pig Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative  
940-150-HFA Horse Anti-Diphtheria Toxin/Toxoid IgG (Fab2) ELISA kit, 96 tests, Quantitative  
940-200-DHG Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests,  
940-210-DHM Human Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests,  
940-220-DMG Mouse Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests,  
940-225-DMM Mouse Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests,  
940-230-DRG Rabbit Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests,  
940-235-DRM Rabbit Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests,  
940-245-DKM Monkey Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative

930-100-TTH Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
930-110-TTM Mouse Anti-Tetanus Toxin/Toxoid Ig's (G+A+M) ELISA kit, 96 tests, Quantitative  
930-120-TMA Mouse Anti-Tetanus Toxin/Toxoid IgA ELISA kit, 96 tests, Quantitative  
930-130-TMG Mouse Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
930-140-TMM Mouse Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative  
930-200-TTR Rabbit Anti-Tetanus Toxin/Toxoid Ig's (G+A+M) ELISA kit, 96 tests, Quantitative  
930-210-TRG Rabbit Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
930-220-TRM Rabbit Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative  
930-310-TGG G. pig Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
930-320-TGM G. pig Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative  
930-410-TKG Monkey Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
930-500-HTG Horse Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative  
930-510-HFA Horse Anti-Tetanus Toxin/Toxoid IgG-Fab2 ELISA kit, 96 tests, Quantitative

Instruction Manual No. M-950-110-AHG

## Human Anti-Adenovirus IgG ELISA KIT Cat. # 950-110-AHG For Detecting Human IgG antibodies against Adenovirus in Serum or Plasma



For In Vitro Research Use Only

  
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INTERNATIONAL**

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Human Anti-Adenovirus IgG ELISA KIT Cat. #950-110-AHG (96 tests)

Kit Components (96 tests)	Cat #
Adenovirus antigen coated strip plate, (8x12 strip or 96 wells) # 950111	1 plate
Anti-Adenovirus IgG Calibrator A (1 U/ml, -ve control) 2 ml #950112A	1 vial
Anti-Adenovirus IgG Calibrator B (10 U/ml, cut-off control) 2 ml #950112B	1 vial
Anti-Adenovirus IgG Calibrator C (35 U/ml, weak positive control) 2 ml #950112C	1 vial
Anti-Adenovirus IgG Calibrator D (150 U/ml, high positive control) 2 ml #950112D	1 vial
Anti-Human IgG-HRP Conjugate, (15 ml) #950-113	1 bottle
Sample Diluent, 60 ml #950110-SD	1 bottle
Wash buffer (10X) 60 ml # 950110-WB	1 bottle
TMB Substrate Solution, 15 ml #950110-TM	1 bottle
Stop Solution, 15 ml # 950110-SS	1 bottle
Plastic Resealable bag for the un-used antigen strips	1
Complete Instruction Manual # M-950-110-AHG	1

**Intended Use**

ADI Human Anti-Adenovirus IgG ELISA Test Kit has been designed for the detection and measurement of specific IgG class antibodies against Adenovirus in serum and plasma. The coated antigen is purified Hexon (Ad5 strain). This kit is for in vitro research use only (RUO).

**Introduction**

The adenovirus is a ubiquitous pathogen of humans and animals. Adenoviruses are characterized by location inside the cell nucleus, common complement-fixing antigens and marked stability to environmental effects. Adenoviruses are endemic in all populations throughout the year. The infection is spread both through the aerial-droplet route and the routes characteristic for intestinal infections. The incubation period is between five and seven days. Adenoviruses mainly infest respiratory and intestinal mucosa, but also the cornea. They are accumulated in the epithelial cells and regional lymph nodes. Adenoviruses cause the widest variety of illnesses of the known respiratory viruses. The adenovirus infection is the most frequently caused viral disease of the respiratory tract among preschool children (types 1 - 5 and 7). Acute diseases of the upper respiratory tract occur predominantly. Pneumonia is the most severe form of adenoviral infection occurring mostly in infants below the age of one. Adenoviruses also cause outbreaks of swimming-pool associated pharyngo conjunctival fever in the summer and epidemics of keratoconjunctivitis of both children and adults. The intestinal form of adenoviral infection occurs mostly in children below the age of one. An acute adenoviral infection can be detected by virus isolation and/or serology. The serologic tests are particularly important because they document actual infection in the patient and can be applied to large scale epidemiologic investigations. The CF and ELISA tests measure predominantly the antibodies directed against the group-specific determinants on the hexon component. The recommended tests for measuring type-specific antibodies are hemagglutinin inhibition and serum neutralization.

The type-specific antigenic determinants of adenoviruses are located at the fibers on the capsid. Because of the ubiquity of the adenoviruses and numerous cross-reactions between related serotypes, sero conversion involving a fourfold or greater rise in antibody infection is necessary to document infection. IgG is the predominant antibody class measured in the serologic tests.

**Equivocal Samples**

If a sample value is in the range from 8U/mL to 12U/mL (close to the cut-off values), it is suggested that the sample be interpreted as equivocal and the test be repeated in duplicate using the same sample or with a new sample taken after 2-4 weeks. Both samples should be measured in parallel in the same run.

**Positive Samples**

All samples with values above the Cut-off are considered positive. Positive control values must be at least twice the values of the Cut-off control for the test to be valid.

**Quality Control**

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. The positive control must show at least double the OD of the cut-off standard. 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, equipments, etc).

**PERFORMANCE CHARACTERISTICS**

**Intra-Assay-Precision** 8.1 %      **Inter-Assay-Precision** 11.3 %

**Analytical Sensitivity** ~1U/mL

**Interferences**

No interferences to bilirubin up to 0.3 mg/mL; Hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL.

**Cross Reactivity**

No cross reactivity to Influenza A and RSV.

**Species Reactivity**

This kit is tested for human samples only. ADI has other kits for monkey, mouse, and other animals.

**Adenovirus Hexon Antigen Specificity**

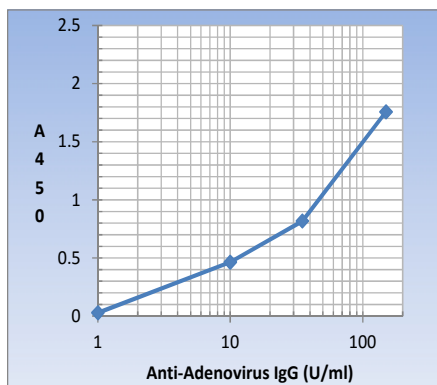
This kit uses human mastadenovirus C, Adv5 or hAd5 hexon purified antigen (952-aa, Adeno hexon C or L3) are relatively highly conserved in various major type (Adenovirus B, D, and F; 77-80%); Most human adenovirus subtypes hAd1-hAd50 show protein conservation 76-80%. Many chimp (Ad1), gorilla (Ad7-9), and simian (Ad1, 7, 18, 22, 24, 25,) and Feline Adenovirus (87). Therefore, high degree of hexon antibody crossreactivity between the same species or across the species is to be expected. We have not confirmed if hAd5 hexon antigen used in the kit actually recognized antibodies to other related adenovirus types and subtypes. Chimp Ad3 vectors are also being tested for Ebola and other vaccines. chAd3 and hAd5 hexon proteins are ~88% conserved. Therefore, ADI's hAd5 hexon antigen kit is expected to react with chAd3 hexon antibodies as well. Human Ad5 or chAd3 hexon show 57-60% conservation with recombinant purified mouse Adenovirus Hexon protein.

**References:** Hierholzer, J. C. et al (1986) Adenoviruses, 527; McMinn PC, et al (1991) Community Outbreak Keratoconjunctivitis in due to Adenovirus 164; 1113; Wadell G., et al (1988) Adenoviridae: The adenoviruses Vol 2: 284; Wigand, R. et al (1982) 18; 169.

## WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	Net A450	Results
A1, A2	<b>Blanks</b>	0.15	-	-
B1, B2	Negative Control A (1 U/ml)	0.054	0.039	
C1, C2	<b>Cut-off Control B (10 U/ml)</b>	<b>0.409</b>	<b>0.464</b>	
D1, D2	Weak Positive Control C (50 U/ml)	0.803	0.788	
D1, D2	Positive Control D (150 U/ml)	1.641	1.626	
E1, E2	<b>Sample 1</b>		0.294	Negative
F1, F2	<b>Sample 2</b>		1.694	Positive
G1, G2	<b>Sample 3</b>		0.694	WeakPositive
H1, HG2	<b>Sample 4</b>		0.594	Equivocal

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



**Positive samples:** If the value of the sample is **higher than the value of the cutoff** standard, that sample should be interpreted as a positive result.

**Negative samples:** For a value **below the cut-off standard** (arbitrary units = 10 U/ml) the sample should be interpreted as a negative result.

### 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 or 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 reassayed.

Adenovirus infections cause approximately 15,000 illnesses per year in basic Army trainees. In the past, US military recruits were vaccinated against two serotypes of adenotypes, with a corresponding decrease in illnesses caused by those serotypes. The vaccine is no longer manufactured, and there are currently no vaccines available to protect against the adenovirus. The new adenovirus vaccine tablets offers protection against two strains of the virus, type 4 and type 7, and is administered in tablet form containing the live virus (32,000 TCID). The tablets are intended to be swallowed whole so they can pass through the stomach intact and then release the virus in the intestines. In clinical trials supported by both the Army and the Navy among other organizations, scientists found the new vaccine provided 99.3 percent protection against febrile respiratory illnesses due to the adenovirus type 4 while stimulating protective levels of antibodies against the adenovirus type 7.

Adenovirus is also used as a vehicle to administer targeted therapy, in the form of recombinant DNA or protein. Specific modifications on fibre proteins are used to target Adenovirus to certain cell types. Adenovirus dodecahedron can qualify as a potent delivery platform for foreign antigens to human myeloid dendritic cells (MDC), and that it is efficiently presented by MDC to M1-specific CD8+ T lymphocytes.

### Adenovirus based vaccines

Replication-defective Adenovirus-transgene induce potent B and T-cell specific immune responses in humans and animals. These vectors are being used for use as carriers of vaccines for a variety of pathogens, including human immunodeficiency virus type 1 (HIV-1), Plasmodium falciparum, Mycobacterium tuberculosis, and Ebola. Since humans commonly come in contact with adenoviruses, which cause respiratory, gastrointestinal and eye infections, majority of patients have already developed neutralizing antibodies which can inactivate the virus before it can reach the target cell. To overcome this problem scientists are currently investigating adenoviruses that infect different species to which humans do not have immunity. For example, chimp Ad3-Ebola vaccine is based upon chAd3 that supposedly had low exposure to human.

### PRINCIPLE OF THE TEST

Alpha Diagnostic's Adenovirus IgG Antibody ELISA Test Kit is based on the principle of the enzyme immunoassay (EIA). Adenovirus 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 Adenovirus antigen takes place. After a 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-human-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.

### PRECAUTIONS

Only for in-vitro use! 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 have to be taken. 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.

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 Proclin-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](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 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

## REAGENTS PREPARATION

1. Dilute Wash buffer 1:10 with water, (60 ml stock in 540 ml distilled water) Store diluted buffer at 4°C for 1 month.

*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).

**Important:** If you have not used this kit before, we recommend to use 1 or 2 strips to run the standards alone to get familiar with the test and not run the risk of making mistakes and lose sample or the whole kit.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **All samples should be diluted 1:101 (5 ul samples in 500 ul sample diluent).** It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate.

1. Label or mark the microtiter well strips to be used on the plate. Dilute the wash buffer with water (1:10).
2. Dispense 100 ul diluent in 1 well to be used as blank. Pipet **100 ul of Prediluted controls, and samples** (diluted 1:101) 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 for 60 min.**
3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 4 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 Humananti-IgG-HRPconjugate** 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 (25-28°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 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°C. Do not touch the bottom of the wells.

## Interpretation of results:

Most of the data presented here for information purpose. Therefore, users are suggested to establish their own reference values.

U/ml	Interpretation
< 8	Negative
8- 12	equivocal
>12	positive

## Expected Values

An in-house study of normal human random samples showed the following results.

Ig isotype	n	Interpretation		
		Positive	Equivocal	Negative
IgG	56	94.6%	1.8%	5.4%