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

Catalog#	ProdDescription
950-100-AHA	Human Anti-Adenovirus (hAd5 hxn) IgA ELISA kit, 96 tests, Quantitative
950-110-AHG	Human Anti-Adenovirus (hAd5 hxn) IgG ELISA kit, 96 tests, Quantitative
950-120-AHM	Human Anti-Adenovirus (hAd5 hxn) IgM ELISA kit, 96 tests, Quantitative
950-130-AMG	Mouse Anti-Human Adenovirus (hAd5 hxn) IgG ELISA kit, 96 tests, Quantitative
950-140-AMM	Mouse Anti-Human Adenovirus (hAd5 hxn) IgM ELISA kit, 96 tests, Quantitative
950-150-AMG	Monkey Anti-Human Adenovirus (hAd5 hxn) IgG ELISA kit, 96 tests, Quantitative
950-155-AMM	Monkey Anti-Adenovirus (hAd5 hxn) IgM ELISA kit, 96 tests, Quantitative
AE-327100-1	Mouse Anti-Adenovirus hexon antibody (hAdVHxn) IgG ELISA Kit, 96 tests,
AE-327110-1	Human Anti-Adenovirus hexon antibody (hAdVHxn) IgG ELISA Kit, 96 tests,
AE-327120-1	Monkey/Chimp Anti-Adenovirus hexon antibody (hAdVHxn) IgG ELISA Kit
950-110-NC	Human Anti-Adenovirus Hexon Protein (hxn) IgG Negative Control Serum
950-110-PC	Human Anti-Adenovirus Hexon Protein (hxn) IgG Positive Control Serum
950-130-MNC	Mouse Anti-Adenovirus Hexon Protein (hxn) IgG Negative Control Serum
950-130-MPC	Mouse Anti-Adenovirus Hexon Protein (hxn) IgG Positive Control Serum
950-150-CN	Chimpanzee Anti-Adenovirus Hexon Protein (hxn) IgG Negative Control Serum
950-150-CP	Chimpanzee Anti-Adenovirus Hexon Protein (hxn) IgG Positive Control Serum
950-150-MM	Monkey (Cynomolgus) Anti-Adenovirus HexonIgG Negative Control Serum
950-150-MP	Monkey (Cynomolgous) Anti-Adenovirus Hexon Protein (hxn) IgG Positive Control
950-150-NC	Baboon Anti-Adenovirus Hexon Protein (hxn) IgG Negative Control Serum
950-150-PC	Baboon Anti-Adenovirus Hexon Protein (hxn) IgG Positive Control Serum
950-150-RN	Monkey (Rhesus) Anti-Adenovirus Hexon Protein (hxn) IgG Negative Control
950-150-RP	Monkey (Rhesus) Anti-Adenovirus Hexon Protein (hxn) IgG Positive Control
ADV5-GNC	Goat Anti-Mouse Adenovirus (MADV) Hexon Protein (hxn) IgG Negative Control
ADV5-GPC	Goat Anti-Mouse Adenovirus (MADV) Hexon Protein (hxn) IgG Positive Control
ADV5-RNC	Rabbit Anti-Mouse Adenovirus (MADV) Hexon Protein (hxn) IgG Negative Control
ADV5-RPC	Rabbit Anti-Mouse Adenovirus (MADV) Hexon Protein (hxn) IgG Positive Control
ADV11-A	Anti-Adenovirus type 2, hexonIgG (reacts with 1-7a, 8, 31, 40-41)
ADV11-BTN	Anti-Adenovirus type 2, hexonIgG-Biotin conjugate
ADV11-FITC	Anti-Adenovirus type 2, hexonIgG-FITC conjugate
ADV11-HRP	Anti-Adenovirus type 2, hexonIgG-HRP conjugate
ADV12-FITC	Monoclonal Anti-Adenovirus (many isotypes) IgG-FITC conjugate
ADV12-M	Monoclonal Anti-Adenovirus (many istoypes) hexonIgG
ADV13-M	Monoclonal Anti-Adenovirus type 40 IgG, aff pure
ADV14-M	Monoclonal Anti-Adenovirus type 41 IgG, aff pure
ADV15-M	Monoclonal Anti-Adenovirus type 40/41 IgG, aff pure
ADV16-M	Monoclonal Anti-Adenovirus hexon (types 1, 5, 8, 27) IgG
ADV17-M	Monoclonal Anti-Adenovirus type (pan, reacts with all human serotypes) IgG
ADV31-M	Monoclonal Anti-Adenovirus type 3 hexon (hxn) IgG, aff pure
ADV51-M	Monoclonal Anti-Adenovirus type 5 hexon (hxn) IgG, aff pure
ADV55-R-10	Human Adenovirus type 5 hexon (Hxn/L3 antigen) purified protein
ADV65-N	Adenovirus (strain Adenoid 6) type 2, semi-pure viral lysate (
ADV66-N	Adenovirus (strain Adenoid 6) type 2 hexons antigens, purified (host Vero cells)
HCLS-17010	293 Cell Slide (Human (embryonal) kidney transformed by sheared human adenovirus 5 (Ad 5) DNA) (5 slides/pk)
MADV11-C	Recombinant (E. Coli) Mouse Adenovirus (MADV) Hexon Protein (hxn) Control
MADV11-S	Rabbit Anti-Mouse Adenovirus (MADV) Hexon (hxn) Protein Antiserum
MADV15-R-10	Recombinant (E. Coli,) Mouse Adenovirus (MADV) Hexon (hxn) Protein

Instruction Manual No. M-950-150-AMG

MonkeyAnti-Adenovirus IgG

ELISA KIT Cat # 950-150-AMG, 96 Tests

For Detecting Monkey IgG antibodies against Adenovirus Hexon in Serum or Plasma

For In Vitro Research Use Only

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Monkey Anti-Adenovirus IgGELISA KIT Cat. #950-150-AMG(96 tests)

Kit Components (96 tests)	Cat #
Adenovirusantigen coated strip plate, (8x12 strip or 96 wells)# 950151	1 plate
Anti-Adenovirus IgG **Cal A** (1 U/ml, -ve control) 2 ml #950152A	1 vial
Anti-Adenovirus IgG **Cal B** (10 U/ml, cut-off control) 2 ml #950152B	1 vial
Anti-Adenovirus IgG **Cal C** (35 U/ml, weak positive control) 2 ml #950152C	1 vial
Anti-Adenovirus IgG **Cal D** (150 U/ml, high positive control) 2 ml #950152D	1 vial
Monkey Adenovirus IgG positive control, (1 ml) #950153PC	1 bottle
Anti-Monkey IgG-HRP Conjugate, (15 ml) #950154	1 bottle
Sample Diluent, 60 ml #950150-SD	1 bottle
Wash buffer (10X) 60 ml # 950150-WB	1 bottle
TMB Substrate Solution, 15 ml #950150-TM	1 bottle
Stop Solution, 15 ml # 950150-SS	1 bottle
Reseal able bag for the un-used antigen strips	1
Complete Instruction Manual # M-950-150-AMG	1

Intended Use

ADI Monkey 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). It has been tested in rhesus, cynomolgous, and baboon samples. 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 thecornea. 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-poolassociated pharyngoconjunctival fever in the summer and epidemics of kerato-conjunctivitis 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 largescale 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, seroconversion 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.

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. Eachlaboratory should use known samples as further controls.

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 Monkey samples only. ADI has other kits for monkey, mouse, and other animals.

Adenovirus Hexon Antigen Specificity

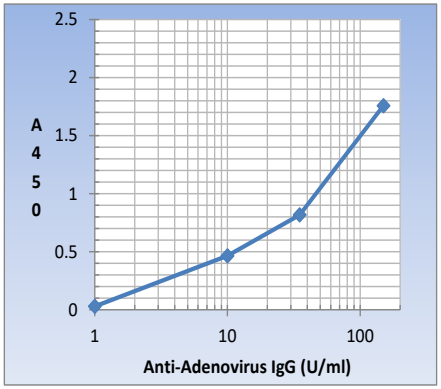
This kit uses human mastadenovirus C, Adv5 or hAd5 hexon purified antigen (952-aa, Adenohexon C or L3) are relatively highly conserved in various major type (Adenovirus B, D, and F; 77-80%); Most human adenovirus subtypes hAd1-hAd50 show protein conservation 76-80%. Many chimp (Ad1), gorilla (Ad7-9), 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, JCet al (1986) Adenoviruses, 527; McMinn PC, et al (1991) Community Outbreak Keratoconjunctivitis in due to Adenvirus 164;1113;Wadell G.,et al (1988) Adenviridae: The adenovirusesVol 2: 284; Wigand, R. et al (1982) 18; 169.

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	Net A450	Results
A1, A2	**Blanks**	0.15	-	-
B1, B2	Negative Control A (1 u/ml)	0.054	0.039	
C1, C2	Cut-off Control B (10 U/ml)	0.409	**0.464**	
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	**Sample 1**		0.294	Negative
F1, F2	**Sample 2**		1.694	Positive
G1, G2	**Sample 3**		0.694	WeakPositive
H1, HG2	**Sample 4**		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 of 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 “Test Procedure” and re-assayed.

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-trasngene 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-Monkey-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 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 1:101 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 4oC 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^{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).*

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 runt 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** ready-to-use calibrators, 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 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 (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^{o}C. Do not touch the bottom of the wells.

Interpretation of results:

Most of the data presented here for information purpose and it is based upon human sample analyses. Therefore, users are suggested to establish their own reference values for monkey samples.

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

Monkey Sample Testing

A random testing of adult rhesus, Cynomolgus, and baboons' sera at 1:100 dilution produced the following results.

	Serum tested at 1:100 dilution (A450 values)							
	1	2	3	4	5	6	7	8
Cyno	0.215	0.161	0.171	0.202	0.139	0.205	0.718	0.235
Rhesus	0172	0.322	0.414	1.030	0.307	0.366	1.249	0.295
Baboon	0662	0.180	0.130	0.169	0.595	0.311	0.487	0.177

As compared to humans, monkey show very low prevalence of pre-existing antibodies to human adenovirus hexon protein. Monkey that are vaccinated with human adenovirus vaccines are expected to produce higher levels of antibodies against the human adenovirus.