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. pertussis Pertactin IgG ELISA kit, 96 tests
960-240-PRG	Rabbit Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-250-PHG	Human Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-260-PMG	Monkey Anti-B. pertussis Pertactin 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-950-120-AHM

Human Anti-Adenovirus IgM
ELISA KIT Cat. # 950-120-AHM
For Detecting Human IgM antibodies against Adenovirus in 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 CHNAGES.

Human Anti-Adenvirus IgM ELISA KIT Cat. # 950-120-AHM (96 tests)

Kit Components (96 tests)	Cat #
Adenovirus antigen coated strip plate, (8x12 strip or 96 wells) # 950-121	1 plate
Adenovirus IgM **Calibrator A** (Negative control), 2 ml #950-122A	1 vial
Adenovirus IgM **Calibrator B (**Cut-off control); 2 ml #950-122B	1 vial
Adenovirus IgM **Calibrator C (**Weak Positive control); 2 ml#950-122C	1 vial
Adenovirus IgM **Calibrator D (**Positive control);2 ml#950-122D	1 vial
Human Anti-Adenovirus Conjugate, (15 ml) #950-123	1 bottle
Sample Diluent, 60 ml #950120-SD	1 bottle
Wash buffer (**10X**) 60 ml # 950120-WB	1 bottle
TMB Substrate Solution, 15 ml #950-120-TM	1 bottle
Stop Solution, 15 ml # 950-120-ST	1 bottle
Plastic foil for covering plate and Reseal able bag for the un-used antigen strips	1
Complete Instruction Manual; M-950-120-AHM	1

Intended Use

ADI Human Anti-Adenovirus IgM ELISA Test Kit has been designed for the detection of specific IgG class antibodies against Adenovirus in serum and plasma. This kit is for in vitro research use only.

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 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 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, 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.

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 ~1 U/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

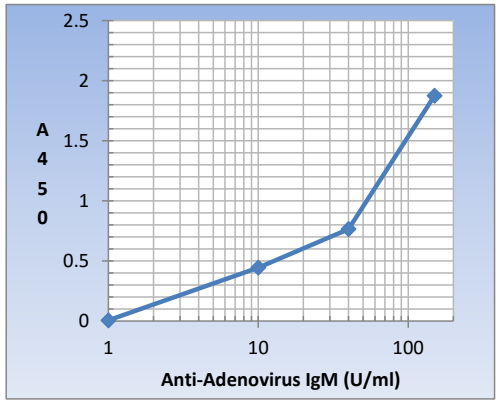
This kit is tested in mouse samples only. It has not been tested in rat, chickens or other animals.

References: Hierholzer, JC et 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 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	**Blanks**	0.15	-	-
B1, B2	Negative Control A	0.054	0.039	
C1, C2	Cut-off Control B	0.409	**0.464**	
D1, D2	Weak Positive Control C	0.803	0.788	
D1, D2	Positive Control D	1.641	1.626	
E1, E2	**Sample 1**		0.294	Negative
F1, F2	**Sample 2**		1.694	Positive
G1, G2	**Sample 3**		0.694	Weak Positive
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 “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.

PRINCIPLE OF THE TEST

Alpha Diagnostic’s Adenovirus IgM 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-touse standards are pipetted into the wells of the microtiter plate. A binding between the IgM 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-IgM 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 IgM 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! 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. 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. 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. 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** 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 Human anti-IgM-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 (25-28oC).

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 tempr. 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 interrption 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. 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
IgM	72	0.0 %	0.0 %	100 %