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-950-120-AHM

Human Anti-Adenovirus IgM ELISA KIT

Cat # 950-120-AHM, 96 Tests

For Detecting Human IgM antibodies against Adenovirus in Serum or Plasma

For In Vitro Research Use Only

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Web Site: www.4adi.com

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

Kit Components (96 tests)	#
Adenovirus antigen coated strip plate, (8x12 strip or 96 wells)# 950121	1 plate
Calibrator A (Negative control) 2 ml #950122A	1 vial
Calibrator B (Cut-off control) 3 ml #950122B	1 vial
Calibrator C (Positive control) 2 ml #950122C	1 vial
Anti-Human IgM-HRP Conjugate, 20 ml #950-123	1 bottle
Sample Diluent, 100 ml #950120-SD	1 bottle
Wash buffer (20X) 50 ml # 950120-WB	1 bottle
TMB Substrate Solution, 15 ml #950120-TM	1 bottle
Stop Solution, 15 ml # 950120-SS	1 bottle
Complete Instruction Manual # M-950-120-AHM	1

Intended Use

ADI Human Anti-Adenovirus IgM ELISA Test Kit has been designed for the detection and measurement of specific IgM class antibodies against Adenovirus in serum and plasma (citrate, heparin). 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 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, 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.

Quality Control

The test results are only valid if the test has been performed following the instructions. All Calibrators 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, equipment's, etc.).

Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value < 0.100
- **Negative Control**: Absorbance value < 0.200 and < Cut-off
- **Cut-off Control:** Absorbance value 0.150 – 1.300
- **Positive Control:** Absorbance value > Cut-off

If these criteria are not met, the test is not valid and must be repeated.

PERFORMANCE CHARACTERISTICS

Precision

Intraassay:	<4.74%
Interassay:	<11.10%
Specificity	97.5%
Sensitivity	90.0%

Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

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 cross reactivity between the same species or across the species is to be expected. We have not confirmed if hAd5 hexon antigen used in the kit 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)

WORKSHEET OF A TYPICAL ASSAY

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

	A450
Calibrator A (Negative Control)	0.003
Calibrator B (Cut off Control)	0.561
Calibrator C (Positive Control)	1.141

CALCULATION OF RESULTS:

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control

0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

Results in Units [U]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = [\text{Units} = \text{U}]$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ U (Units)

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 offer 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 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-to-use 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, 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 15 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! 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).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (citrate, 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. Heat inactivation of samples is not recommended. For the performance of the test the samples (not the standards) have to be diluted 1:100 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

REAGENTS PREPARATION

1. **Dilute Wash buffer (20X)** 1:19 with distilled water. (**e. g. 10 mL Washing Buffer + 190 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.

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 12 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 using 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 1:100 (5 ul samples in 500 ul sample diluent)**. **Calibrators provided with the kit are ready-to-use**. It is recommended to prepare a parallel replica plate 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 (1:19).

2. Dispense **100 ul** diluent in 1 well to be used as blank. Pipet **100 ul of** ready-to-use standards/controls, and samples (diluted 1:100) 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 37°C 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-human 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 (20-25ºC).

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 **15 minutes** at room temp. (20-25 °C) in the dark. 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 30 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. Therefore, users are suggested to establish their own reference values.

U	Interpretation
10	Cut-off
< 9	Negative
9-11	Equivocal
>11	Positive