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

950-100-AHA Human Anti-Adenovirus IgA ELISA kit, 96 tests, Quantitative
950-130-AMG Human Anti-Adenovirus IgG ELISA kit, 96 tests, Quantitative
950-120-AHM Human Anti-Adenovirus IgM ELISA kit, 96 tests, Quantitative

AE-320500-1 Mouse Anti-Zaire Ebola virus Nucleoprotein (NP) IgG ELISA Kit, 96 tests, Quantitative
AE-320510-1 Mouse Anti-Zaire Ebola virus Nucleoprotein (NP) IgM ELISA Kit, 96 tests, Quantitative
AE-320520-1 Human Anti-Zaire Ebola virus Nucleoprotein (NP) IgG ELISA Kit, 96 tests, Quantitative
AE-320530-1 Human Anti-Zaire Ebola virus Nucleoprotein (NP) IgM ELISA Kit, 96 tests, Quantitative
AE-320540-1 Rabbit Anti-Zaire Ebola virus Nucleoprotein (NP) IgG ELISA Kit, 96 tests, Quantitative
AE-320550-1 Monkey/Chimp Anti-Zaire Ebola virus Nucleoprotein (NP) IgG ELISA Kit, 96 tests, Quantitative
AE-320560-1 Monkey/Chimp Anti-Zaire Ebola virus Nucleoprotein (NP) IgM ELISA Kit, 96 tests, Quantitative

AE-320600-1 Mouse Anti-Zaire Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-320610-1 Mouse Anti-Zaire Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-320620-1 Human Anti-Zaire Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-320630-1 Human Anti-Zaire Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-320640-1 Rabbit Anti-Zaire Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-320650-1 Monkey/Chimp Anti-Zaire Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-320650-PC Monkey/Chimp Anti-Zaire Ebola virus glycoprotein (GP) IgG positive control
AE-320660-1 Monkey/Chimp Anti-Zaire Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-320670-1 Dog Anti-Zaire Ebola virus glycoprotein IgG ELISA Kit, 96 tests, Quantitative
AE-320680-1 Pig Anti-Zaire Ebola virus glycoprotein IgG ELISA Kit, 96 tests, Quantitative

AE-320700-1 Mouse Anti-Zaire Ebola virus VP40 IgG ELISA Kit, 96 tests, Quantitative
AE-320710-1 Mouse Anti-Zaire Ebola virus VP40 IgM ELISA Kit, 96 tests, Quantitative
AE-320720-1 Human Anti-Zaire Ebola virus VP40 IgG ELISA Kit, 96 tests, Quantitative
AE-320730-1 Human Anti-Zaire Ebola virus VP40 IgM ELISA Kit, 96 tests, Quantitative
AE-320740-1 Rabbit Anti-Zaire Ebola virus VP40 IgG ELISA Kit, 96 tests, Quantitative
AE-320750-1 Monkey/Chimp Anti-Zaire Ebola virus VP40 IgG ELISA Kit, 96 tests, Quantitative
AE-320760-1 Monkey/Chimp Anti-Zaire Ebola virus VP40 IgM ELISA Kit, 96 tests, Quantitative

AE-320800-1 Zaire Ebola Virus Glycoprotein (EBOV GP antigen) ELISA Kit, 48 tests, Quantitative
AE-320800-96 Zaire Ebola Virus Glycoprotein (EBOV GP antigen) ELISA Kit, 96 tests, Quantitative
AE-320805-RT-10 Zaire Ebola Virus antigen (GP) rapid test (visual results in 2-10 mins), 10 cassettes/pk
AE-320810-1 Humanized (plant expressed) Anti-Ebola GP IgGs ELISA kit, 96 tests, Quantitative
AE-320815-1 Anti-Humanized Ebola GP IgGs (Plant expressed) (Anti-drug antibody/ADA) ELISA kit, 96 tests,
AE-320880-RT-25 Humanized Ebola IgG (humanized IgGs expressed in tobacco or other plants) rapid test (results in 2-10 min), 25 cassettes/pk

AE-321600-1 Mouse Anti-Sudan Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-321610-1 Mouse Anti-Sudan Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-321620-1 Human Anti-Sudan Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-321630-1 Human Anti-Sudan Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-321640-1 Rabbit Anti-Sudan-Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-321650-1 Monkey/Chimp Anti-Sudan Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-321660-1 Monkey/Chimp Anti-Sudan Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative

AE-322600-1 Mouse Anti-Marburg (Angola) virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-322610-1 Mouse Anti-Marburg (Angola) glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-322620-1 Human Anti-Marburg (Angola) glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-322630-1 Human Anti-Marburg (Angola) glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-322640-1 Rabbit Anti-Marburg (Angola) glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-322650-1 Monkey/Chimp Anti-Marburg (Angola) glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-322660-1 Monkey/Chimp Anti-Marburg (Angola) glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative

AE-323620-1 Human Anti-Reston Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative

AE-324620-1 Human Anti-Bundibugyo virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative

AE-325600-XH Human Anti-Zaire+Sudan+Reston+ Bundibugyo Glycoproteins combo IgG ELISA Kit, 96 tests,
AE-325600-XM Monkey Anti-Zaire+Sudan+Reston+ Bundibugyo Glycoproteins combo IgG ELISA Kit, 96

AE-327100-1 Mouse Anti-Adenovirus hexon antibody (hAdV Hxn) IgG ELISA Kit, 96 tests, Quantitative
AE-327110-1 Human Anti-Adenovirus hexon antibody (hAdV Hxn) IgG ELISA Kit, 96 tests, Quantitative
AE-327120-1 Monkey/Chimp Anti-Adenovirus hexon antibody (hAdV Hxn) IgG ELISA Kit, 96 tests, Quantitative
AE-327200-1 Mouse Anti-VSV Indiana Matrix (M) antibody (VSVIM) IgG ELISA Kit, 96 tests, Quantitative
AE-327210-1 Human Anti-VSV Indiana Matrix (M) antibody (VSVIM) IgG ELISA Kit, 96 tests, Quantitative
AE-327300-1 Mouse Anti-VSV Indiana Matrix (M) antibody (VSVIM) IgG ELISA Kit, 96 tests, Quantitative
AE-327310-1 Human Anti-VSV Indiana Glycoprotein (GP) antibody (VSVIG) IgG ELISA Kit, 96 tests, Quantitative
AE-327320-1 Monkey/Chimp Anti-VSV Indiana Glycoprotein (GP) antibody (VSVIG) IgG ELISA Kit, 96 tests, Quantitative

Instruction Manual No. M-950-130-AMG

Mouse Anti-Adenovirus IgG

ELISA KIT

Cat. # 950-130-AMG, 96 Tests

For Detecting IgG antibodies against Adenovirus in Mouse Serum or Plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Mouse Anti-Adenovirus IgG ELISA KIT Cat. # 950-130-AMG (96 tests)

Kit Components (96 tests)	Cat #
Adenovirus antigen coated strip plate, (8x12 strip or 96 wells) # 950131	1 plate
Adenovirus IgG **Calibrator A, Negative Control;** 2 ml #95132A	1 vial
Adenovirus IgG **Calibrator B, Cut-Off Control;** 3 ml #950132B	1 vial
Adenovirus IgG **Calibrator C, Positive Control;** 2 ml #950132C	1 vial
Anti-Mouse IgG-HRP Conjugate, (20 ml) #950133	1 bottle
Sample Diluent, 100 ml #950130-SD	1 bottle
Wash buffer (20X) 50 ml # 950130-WB	1 bottle
TMB Substrate Solution, 15 ml #950130-TM	1 bottle
Stop Solution, 15 ml # 950130-ST	1 bottle
Complete Instruction Manual # M-950-130-AMG	1

Intended Use

ADI Mouse Anti-Adenovirus IgG ELISA Test Kit has been designed for the detection of IgG class antibodies against Adenovirus in mouse serum and plasma. **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 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 biquity of the adenoviruses and numerous cross-reactions between related serotypes, seroconversion involving a fourfold or greater rise in antibody

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.

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. Approx. 95% of normal population is positive for adenovirus IgG.
For **mouse samples**, we suggest that the users define their own limits of vaccinated or infected animals. A limited study of mouse samples (non-vaccinated, non-infected, and infected) for adenovirus IgG using this ELISA kit produced the following results

Samples	Healthy animals Non-vaccinated	Naturally infected
1:100 dilution	<0.10-0.20 ((n=10)	0.6-2.1 (n=3)

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 **Mouse Adenovirus positive control** must show A450 greater than 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.).

PERFORMANCE CHARACTERISTICS

Intra-Assay-Precision: 5.22 %; **Inter-Assay-Precision** 10.86 %

Diagnostic Sensitivity: 100% **Diagnostic Specificity** 90.91%

Interferences:
No interferences to bilirubin up to 0.5 mg/mL; Hemoglobin up to 10.0 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. For human studies, we have separate ELISA kits.

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	A450
A1, A2	**Blanks**	-
B1, B2	Calibrator A	0.003
C1, C2	Calibrator B	0.561
D1, D2	Calibrator C	1.141
D1, D2	Positive Control	1.641

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, the sample should be interpreted as a negative result.

Interpretation of Results in Units:

The information is- based upon human samples.

Negative **<9 U**

Equivocal **9-11 U**

Positive Samples **>11 U**

Cut-Off **10 U**

An acute adenoviral infection can be detected by virus isolation and/or serology. The infection is necessary to document infection. IgG is the predominant antibody class measured in the serologic tests.

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.

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-Mouse-IgG 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 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! 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 must be taken. Serum and reagent spills must be wiped off with a disinfecting solution (e.g., sodium hypochlorite, 5%) and have to be disposed of properly. All reagents must 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 must 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 must 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.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI web site.
TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

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 1:100 with ready-to-use sample diluent (e.g. 10 µL serum + 1000 µL sample diluent).

Mouse Sample Dilution

We recommend testing for normal or non-immunized samples at 1:100 dilution. However, vaccinated samples be tested at several dilutions (1:500, 1:2000 etc) to determine the antibody levels that are within the detection range of the kit.

REAGENTS PREPARATION:

1. **Dilute Wash buffer** (20X) 1 + 19; e. g. 10 mL Washing Buffer + 190 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 12 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 using 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 1:100 (10 ul sample in 1000 ul sample diluent)**. **Dilute the wash buffer with water (1:19)**. 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.

2. Dispense **100 ul diluent** in 1 well to be used as blank. Pipet **100 ul ready-to-use** calibrators, 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 minutes.**

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 Mouse anti-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 temperature (20-25°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 **15 minutes** at room temperature (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°C. Do not touch the bottom of the wells.