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

Measles

Catalog#	Product Description
530-100-HMG	Human Anti-Measles IgG ELISA kit, 96 tests, Quantitative
530-110-HMM	Human Anti-Measles IgM ELISA kit, 96 tests, Quantitative
530-120-HMA	Human Anti-Measles IgA ELISA kit, 96 tests, Quantitative
530-130-MMG	Mouse Anti-Measles IgG ELISA kit, 96 tests, Quantitative
530-140-MMM	Mouse Anti-Measles IgM ELISA kit, 96 tests, Quantitative
530-150-MMA	Mouse Anti-Measles IgA ELISA kit, 96 tests, Quantitative
MESL11-A	Anti-Measles (Rubeola/Edmonston strain) Virus IgG
MESL12-M	Monoclonal Anti-Measles (Rubeola/Edmonston strain) Virus IgG
MESL15-N-500	Measles (Rubeola) Virus (Edmonston) proteins/antigen extract
RP-1612	Recombinant (E.Coli) purified Measles virus Large Polymerase (2059-2183)
RP-1613	Recombinant (E.Coli) purified Measles virus Large Polymerase (58-149)
RP-651	Recombinant (E.Coli) Measles Virus Large Polymerase (58-149)
RP-653	Recombinant (E.Coli) Measles Virus Large Polymerase (2059-2183)
RP-654	duplicate entry same as #RP-1611; Recombinant Measles Virus Nucleocapsid
RP-655	Recombinant (E.Coli) Measles Virus Hemagglutinin Mosaic (1-30,115-150,379-410)

Mumps

520-100-HMG	Human Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative
520-110-HMM	Human Anti-Mumps Virus (parotitis) IgM ELISA, 96 tests, Quantitative
520-120-HMA	Human Anti-Mumps Virus (parotitis) IgA ELISA, 96 tests, Quantitative
520-130-MMG	Mouse Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative
MUMS11-S	Anti-Mumps virus (Enders) Virus antiserum
MUMS11-SB	Anti-Mumps virus (Enders) Virus antiserum
MUMS12-M	Monoclonal Anti-Mumps virus (Enders) Virus IgG
MUMS15-N-500	Mumps virus (Enders) proteins/antigens extract
510-100-HRG	Human Anti-Rubella Virus IgG ELISA kit, 96 tests, Quantitative
510-110-HRM	Human Anti-Rubella Virus IgM ELISA kit, 96 tests, Quantitative
510-120-MRG	Mouse Anti-Rubella Virus IgG ELISA kit, 96 tests, Quantitative
510-130-MRM	Mouse Anti-Rubella Virus IgM ELISA kit, 96 tests, Quantitative

Rubella

RP-1413	Recombinant (E.Coli) Rubella Virus E1 Mosaic protein
RP-1414	Recombinant (E.Coli) Rubella Virus E2 protein
RP-1415	Recombinant (E.Coli) Rubella Virus Capsid C protein
RUBL11-A	Anti-Rubella virus (HPV77 strain) IgG, unlabeled
RUBL11-BTN	Anti-Rubella virus (HPV77 strain) IgG-Biotin conjugate
RUBL11-FITC	Anti-Rubella virus (HPV77 strain) IgG-FITC conjugate
RUBL11-HRP	Anti-Rubella virus (HPV77 strain) IgG-HRP conjugate
RUBL12-M	Monoclonal Anti-Rubella virus (HPV72) E2 IgG, aff pure
RUBL13-M	Monoclonal Anti-Rubella virus envelop protein E1 IgG, aff pure
RUBL14-M	Monoclonal Anti-Rubella virus envelop protein E2 IgG, aff pure
RUBL15-M	Monoclonal Anti-Rubella virus capsid protein IgG, aff pure
RUBL15-N-500	Rubella virus (HPV77 strain) proteins/antigens extract
RUBL16-M	Monoclonal Anti-Rubella virus core protein IgG, aff pure
RUBL17-M	Monoclonal Anti-Rubella virus structural glycoprotein E1 IgG, aff pure

Instruction Manual No. M-AE-310620-1

Hantavirus Hantaan IgG ELISA KIT

Cat. # AE-310620-1, 96 Tests

For the qualitative determination of IgG antibodies against the serotype Hantaan of Hantavirus in Human Serum or plasma

For In Vitro Research Use Only

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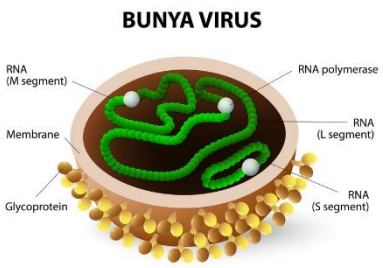
Web Site: www.4adi.com

Kit Components (96 tests)	
recombinant Hantaan NP antigen coated strip plate, (8x12 strip or 96 wells) # 310621-P	1 plate
Positive Control IgG, 2 mL #310622P	1 vial
Cut Off Control IgG, 3 mL #310622CP	1 vial
Negative Control IgG, 2 mL #310622N	1 vial
Anti-IgG Enzyme Conjugate, 20 mL #310623	1 vial
Diluent Buffer, 100 ml #310620-SD	1 bottle
Wash buffer (20X) 50 ml #310620-WB	1 bottle
TMB Substrate Solution, 15 ml #310620-TM	1 bottle
Stop Solution, 15 ml # 310620-ST	1 bottle
Complete Instruction Manual, M-AE-310620-1	1

Intended Use

ADI Hantavirus IgG ELISA Test Kit is an indirect ELISA to test the presence of IgG class antibodies to Hantavirus NP in human serum or plasma. The kit contains highly purified recombinant NP as an antigen and there are no virus or virus-extracted antigens in the kits. **For research use only (RUO), not for diagnosis, cure or prevention of the disease**.

Introduction



Hantavirus is named for the Hantan River area in South Korea where an early outbreak occurred in 1970. Hantaviruses (HTNV) are single-stranded, enveloped, negative sense RNA viruses in the Bunyaviridae family. HTNV normally infect rodents. Humans may become infected with hantaviruses through contact with rodent urine, saliva, or feces. Some strains of hantaviruses cause potentially fatal diseases in humans, such as hantavirus hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS)—also known as hantavirus cardiopulmonary syndrome (HCPS), a "rare respiratory illness associated with the inhalation of aerosolized rodent excreta (urine and feces) contaminated by hantavirus particles.

The Bunyaviridae family is divided into five genera: Orthobunyavirus, Nairovirus, Phlebovirus, Tospovirus, and Hantavirus. Like all members of this family, hantaviruses have genomes comprising three negative-sense, single-stranded RNA segments. Members of other Bunyaviridae family genera are generally arthropod-borne viruses, but hantaviruses are thought to be transmitted to humans mainly through inhalation of aerosolized rodent excreta or rodent bites.

Quality Control

Note: The test results are only valid if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards /laws. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

PERFORMANCE CHARACTERISTICS

In an in-house study, apparently healthy subjects showed the following results:

Precision		Range (Q)	Mean Recovery %	Range CV %
Intra-Assay (n=20)	IgG	**0.7- 2.4**	**3.9**	**2.7- 6.3**

It is recommended that each laboratory establishes its own range of normal values.

Specificity:
The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 96.59 % (95 % confidence interval: 90.36 % - 99.29 %).

Sensitivity:
The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 99.16 % (95 % confidence interval: 95.41 % - 99.98 %).
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
Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions.

SPECIMEN COLLECTION AND STORAGE

Human serum

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material. Human serum must be used as sample material for the Hantavirus (Hantaan) IgG/IgM ELISA.

Storage: 2-8°C ≤ -20°C (Aliquots) Keep away from heat or direct sunlight.
Avoid **Stability**: 5 days 12 months repeated freeze-thaw cycles.

Evaluation of Results

Cut-Off	10 U	
Positive	**> 11 U**	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	**9 – 11 U**	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.
Negative	**< 9 U**	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.

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.

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)



HTNV RNA segments designated S (small), M (medium), and L (large). The S RNA encodes the nucleocapsid (N) protein. The M RNA encodes a polyprotein that is cotranslationally cleaved to yield the envelope glycoproteins Gn (formerly G1) and Gc (formerly G2). The L RNA encodes the L protein, which functions as the viral transcriptase/replicase. Within virions, the genomic RNAs of hantaviruses are thought to complex with the N protein to form helical nucleocapsids. These are composed of many copies of the nucleocapsid protein N, which interact with the three segments of the viral genome to form helical structures. The virally encoded RNA polymerase is also found in the interior. By mass, the virion is greater than 50% protein, 20-30% lipid and 2-7% carbohydrate. There is no vaccine for hantavirus. A vaccine known as **Hantavax**, an inactivated vaccine that may not be effective against European hantaviruses like the Puumala (PUUV) virus. The vaccine is considered important as acute hantavirus infections are responsible for significant morbidity and mortality worldwide. It is estimated that about 1.5 million cases and 46,000 death happened in China from 1950 to 2007. The number of cases is estimated at 32,000 in Finland from 2005 to 2010 and 90,000 in Russia from 1996 to 2006.

The first hantavirus vaccine was obtained from formalin inactivated HTNV grown in rodent brain cells and adjuvanted in Alum. It is estimated that about two million doses of rodent brain or cell-culture derived vaccine are given in China every year. Other vaccines are being tested.

PRINCIPLE OF THE TEST

Hantavirus IgG test kit is based on the principle of the enzyme immunoassay (EIA). The microtiter plate is coated with recombinant nucleo-capsid protein of Hantaan virus. For determination of IgG antibodies, during the incubation period specific antibodies against the recombinant Hantaan antigen are bound to the solid phase. After washing, the specific IgG antibodies are detected with peroxidase-conjugated anti human IgG-antibodies respectively. Addition of substrate solution results in a color reaction, which is proportional to the bound specific antibody content. The absorbance is then measured photometrical.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. distilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

PRE-TEST SETUP INSTRUCTIONS:

Allow kit to reach room temperature (18-25°C). Buffer concentrates may contain salt crystals which dissolve quickly at 37°C. Let buffer cool to room temperature (18-25°C) before starting the test.

Preparation of concentrated components (Examples for 32 wells): Note: Dilute required volumes of reagents directly before use!

Dilute Wash buffer (20X) 1:19 with distilled water. (**e. g. 10 mL Washing Buffer + 190 mL distilled water.**) Store diluted buffer at **4°C** for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.

Dilution of samples:

IgG samples to be diluted generally with diluted diluent buffer.

	with	Relation	Remarks
IgG	Diluent Buffer (diluted)	1:100	e.g. 10 µl Sample + 1 mL Diluent

Note: Undiluted samples can be stored at -20 °C for several months.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. 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.
2. Dispense **100 ul** undiluted negative, positive and reference controls as well as diluted sera into each well. Cover the plate, mix gently for 5-seconds and **incubate at room temp for 60 mins at 37 °C**.
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 Enzyme Conjugate IgG** into each well. Mix gently for 5-10 seconds. Cover the plate and **incubate** for **30 mins at (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 20-25 °C**. (Note: For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles
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.

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. 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.

LIMITATIONS OF THE PROCEDURE:

The following substances do not have a significant effect on the test results up to the concentration stated below:

Hemoglobin	**5 mg/mL**
Bilirubin	**0.625 mg/mL**
Triglyceride	**91 mg/mL**

PROCEDURE NOTES:

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration.
8. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
9. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.