Hantavirus IgG ELISA Kit (Human) (OKNX00138)

Instructions for use

For the qualitative detection of Anti-Hantavirus IgG in human serum.

This product is intended for research use only.

Lot to lot kit variations can occur. Use the kit manual which has been provided along with the kit packaging.

Table of Contents

1. Background

Principle

Aviva Systems Biology Hantavirus IgG ELISA Kit (Human) (OKNX00138) is based on standard reverse capture sandwich enzyme-linked immuno-sorbent assay technology. Hantavirus antigen has been pre-coated and blocked in a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and washed. An HRP conjugated detector antibody specific for Human IgG is added, incubated and followed by washing. An enzymatic reaction is produced through the addition of substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration is read by absorbance at 450 nm and is qualitatively proportional to the amount of sample anti-Hantavirus IgG captured the in well.

Background

Hantaviruses are negative sense RNA viruses in the Bunyaviridae family. Humans may be infected with Hantaviruses through urine, saliva or contact with rodent waste products. Some Hantaviruses cause potentially fatal diseases in humans, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS), but others have not been associated with human disease. Human infections of Hantaviruses have almost entirely been linked to human contact with rodent excrement, but recent human-to-human transmission has been reported with the Andes virus in South America. Hantavirus has an incubation time of two to four weeks in humans before symptoms of infection occur. The symptoms of HFRS can be split into five phases:

• Febrile phase: Symptoms include fever, chills, sweaty palms, diarrhea, malaise, headaches, nausea, abdominal and back pain, respiratory problems such as the ones common in influenza virus infection, as well as gastro-intestinal problems. These symptoms normally occur for three to seven days and arise about two to three weeks after exposure.

• Hypotensive phase: This occurs when the blood platelet levels drop and symptoms can lead to tachycardia and hypoxemia. This phase can last for 2 days.

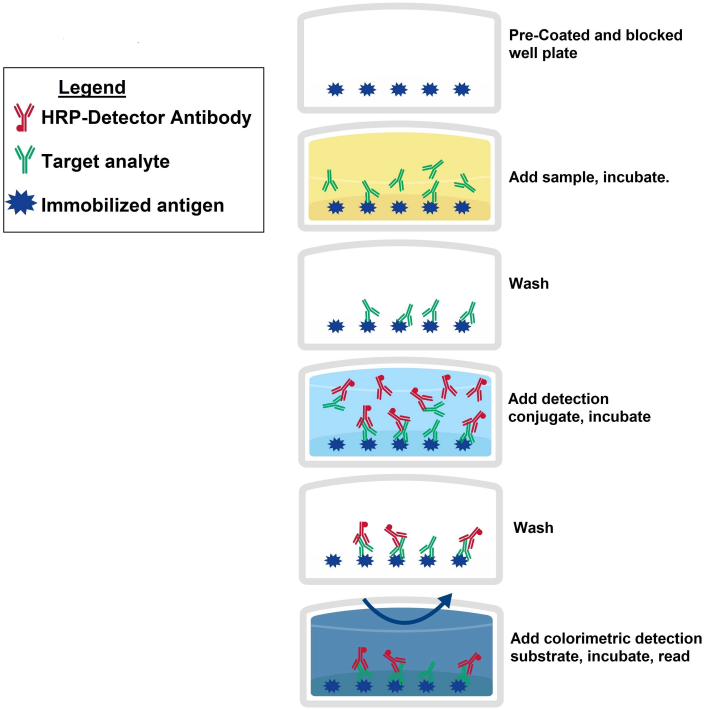
• Oliguric phase: This phase lasts for three to seven days and is characterized by the onset of renal failure and proteinuria occurs.

• Diuretic phase: This is characterized by diuresis of three to six liters per day, which can last for a couple of days up to weeks.

• Convalescent phase: This is normally when recovery occurs and symptoms begin to improve.

Regions especially affected by HFRS include China, the Korean Peninsula, Russia (Hantaan, Puumala and Seoul viruses), and northern and western Europe (Puumala and Dobrava virus).

2. Assay Summary



3. Storage and Stability

• Upon receipt store kit at 4°C for 6 months.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description		Quantity	Storage Conditions
Hantavirus IgG Microplate		96 Wells (12 x 8 Well strips)	4°C for 6 Months
Sample Diluent	White Cap	1 x 100 mL	
Stop Solution	Red Cap	1 x 15 mL	
20X Wash Solution	White Cap	1 x 50 mL	
Anti-Human IgG HRP Conjugate	Black Cap	1 x 20 mL	
TMB Substrate	Yellow Cap	1 x 15 mL	
Hantavirus IgG Cut-Off Control	Green Cap	1 x 3 mL	
Hantavirus IgG Positive Control	Red Cap	1 x 2 mL	
Hantavirus IgG Negative Control	Blue Cap	1 x 2 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

• The following reagents are provided at ready to use concentrations and require no preparation:

- Sample Diluent
- Stop Solution
- Anti-Human IgG HRP Conjugate
- TMB Substrate
- Hantavirus IgG Cut-Off Control
- Hantavirus IgG Positive Control
- Hantavirus IgG Negative Control

8.1 1X Wash Buffer

8.1.1 If crystals have formed in the 20X **Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.1.2 For 500 mL, add 25 mL contents of the 20X **Wash Buffer** bottle to 480 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.1.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.1.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.2 Microplate Preparation

• Micro-plates are provided ready to use and do not require rinsing or blocking.

• Unused well strips should be returned to the original packaging, sealed and stored at 4°C.

• Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C.Avoid repeated freeze-thaw cycles.

• Samples not indicated in the manual must be tested to determine if the kit is valid.

• Prepare samples as follows:

• Serum - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• Plasma - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

9.2 Sample Dilution (1:100)

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

• Dilute samples using **Sample Diluent**.

• Mix diluted samples gently and thoroughly.

• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

The suggested 100-fold dilution can be achieved by adding 10 µL sample to 990 µL of **Sample Diluent**.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for inter- and intra-assay reproducibility will be observed when incubation at 37°C as indicated in the procedure below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch. Allow additional wells to include the following (recommended in duplicate):

10.1.1 Substrate Blank

10.1.2 Negative Control

10.1.3 Cut-Off Control

10.1.4 Positive Control

10.2 Add 100 µL of the **1:100 diluted samples**, **Positive Control, Cut-Off Control** or **Negative Control** to test wells of the Hantavirus IgG Microplate. At least two replicates are recommended.

10.3 Cover the plate with the well plate lid and incubate at 37±1°C for 60±5 minutes.

10.4 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Wash plate 3 times with **1X Wash Buffer** as follows:

10.6.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.6.2 Allow to soak for ~ 5 seconds.

10.6.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.6.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6.5 Repeat steps 10.6.1 through 10.6.4 **two** more times.

10.7 Add 100 µL of the **1X Anti-Human IgG HRP-Conjugate** to all the wells.

10.8 Cover with the well-plate lid and incubate at room temperature for 30 minutes.

10.9 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.10 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.11 Repeat the wash in step 10.6.

10.13 Add 100 µL of **TMB Substrate** to each well and incubate at room temperature **in the dark** for 15 minutes.

10.14 Add 100 µL of **Stop Solution** to each well in the same order as that of step 10.13.

10.15 Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.14. If wavelength correction is available, set to 620 nm.

11. Calculation of Results

A positive or negative Hantavirus IgG determination is derived by comparing the test samples to the **Positive** and **Negative Controls**.

11.1 Calculation of Results

- The **Cut-Off** is the mean 450 nm absorbance (A_{450}) value of the **Cut-Off Control** sample determinations.
- The Test Specimen measurement is the mean of the replicate A_{450} measurements.

11.2 Run Validation Criteria

For an assay to be considered valid, the following criteria must be met:

Substrate blank: Absorbance value < 0.100.

Hantavirus IgG Negative control: Absorbance value < 0.200 and < Cut-Off

Hantavirus IgG Cut-off Control: Absorbance value 0.150 – 1.30

Hantavirus IgG Positive control: Absorbance value > Cut-Off

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

11.3 Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	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 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

12 Typical Expected Data

12.1 Precision

Replicate samples were measured to assess inter- and intra-assay reproducibility.

Metric	Sample Type	n=	Mean	CV%
Inter-Assay	Negative Serum	24	5.04	8.44
Inter-Assay	Positive Serum	24	24.74	4.2
Inter-Assay	Positive Serum	24	29.89	4.8
Intra-Assay	Negative Serum	24	0.315	10.29
Intra-Assay	Positive Serum	24	1.283	5.68
Intra-Assay	Positive Serum	24	0.731	5.59

12.2 Specificity

Specificity is determined as the probability of the assay indicating a negative score in samples absent of the specific analyte: 98.3%

12.3 Sensitivity

Sensitivity is determined as the probability of the assay indicating a positive score in samples with the specific analyte present: >95.9%

12.4 Cross-Reactivity

Cross reactions with antibodies against other pathogens, especially viruses, can not be excluded

13 Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

Aviva Systems Biology, Corp.
5754 Pacific Center Blvd, Suite 201
San Diego, CA 92121

Phone: 858-552-6979
Toll Free: 888-880-0001
Fax: 858-552-6975

Technical support: techsupport@avivasysbio.com

China

Beijing AVIVA Systems Biology
6th Floor, B Building, Kaichi Tower
#A-2 Jinfu Road.
Daxing Industrial Development Zone
Beijing, 102600, CHINA

Phone: (86)10-60214720
Fax: (86)10-60214722
E-mail: support@avivasysbio.com.cn

中国地址：北京大兴工业开发区金辅路甲 2 号凯驰大厦 B 座 6 层 (102600)
电话: 010-60214720/21
传真: 010-60214722

产品售前咨询及销售: sales@avivasysbio.com.cn
售后及技术支持: support@avivasysbio.com.cn