

**NovaLisa®**

# Hantavirus IgG

**ELISA**

**CE**

**Only for in-vitro diagnostic use**

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Product Number:      HANG0670 (96 Determinations)

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## ENGLISH

### 1. INTRODUCTION

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 may lead to serious diseases in humans, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS).

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).

Species	Disease	Symptoms (e.g.)	Transmission route
Puumala virus  Dobrava virus  Hantaan virus  Seoul virus	Hemorrhagic fever with renal syndrome (HFRS)	Initial: suddenly occurring symptoms like intense headache, back and abdominal pain, fever, chills, nausea, and blurred vision.  Late: low blood pressure, acute shock, vascular leakage, and acute kidney failure	After exposure to aerosolized urine, droppings, or saliva of infected rodents or their nests (airborne transmission).  Also by direct contact with these materials to broken skin or onto mucous membranes.
Andes virus  Sin-Nombre-virus  (New world strains)	Hantavirus pulmonary syndrome (HPS)	Initial: universal symptoms include fatigue, fever and muscle aches, especially in the large muscle groups - thighs, hips, back, and sometimes shoulders. There may also be headache, dizziness, chills, and abdominal problems, such as nausea, vomiting, diarrhea, and abdominal pain.  Late: coughing and shortness of breath, lungs fill with fluid.	Bites by infected rodents.  Human to human transmission can not be excluded (for New World strains).

The presence of pathogen or infection may be identified by:

- PCR
- Serology (e. g. ELISA)

### 2. INTENDED USE

The Hantavirus IgG ELISA is intended for the qualitative determination of IgG antibodies against Hantavirus in human serum or plasma (citrate or heparin).

### 3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

## 4. MATERIALS

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### 4.1. Reagents supplied

- **Hantavirus Coated Microplate (IgG):** 12 breakapart 8-well snap-off strips coated with recombinant Hantavirus antigens in resealable aluminium foil.
- **IgG Sample Diluent:** 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M); pH 7.2 ± 0.2; for washing the wells; white cap.
- **Hantavirus anti-IgG Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibody to human IgG; in phosphate buffer (10 mM); coloured blue, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1%; ready to use; yellow cap; < 5% NMP.
- **Hantavirus IgG Positive Control:** 1 bottle containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Hantavirus IgG Cut-off Control:** 1 bottle containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Hantavirus IgG Negative Control:** 1 bottle containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

### 4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

### 4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes

## 5. STABILITY AND STORAGE

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Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

## 6. REAGENT PREPARATION

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It is very important to bring all reagents and samples to room temperature (20...25 °C) and mix them before starting the test run!

### 6.1. Coated Microplate

The break-apart snap-off strips are coated with recombinant Hantavirus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

### 6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20...25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

### 6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

## 7. SAMPLE COLLECTION AND PREPARATION

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Use human serum or plasma (citrate or heparin) samples with this assay. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Heat inactivation of samples is not recommended.

## 7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

## 8. ASSAY PROCEDURE

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### 8.1. Test Preparation

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to  $37 \pm 1$  °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour  $\pm$  5 min at  $37 \pm 1$  °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!  
Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

### 8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

**Measure the absorbance** of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

## 9. RESULTS

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### 9.1. Run Validation Criteria

In order for an assay to be considered valid, 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.

### 9.2. 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

### 9.2.1. Results in Units [NTU]

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

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

### 9.3. 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 <b>negative</b> .
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.		

#### 9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

## 10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

### 10.1. Precision

Intraassay	n	Mean (E)	Cv (%)
#1	24	0.450	3.61
#2	24	1.333	6.41
#3	24	1.264	4.78
Interassay	n	Mean (NTU)	Cv (%)
#1	12	27.44	5.34
#2	12	25.44	8.15
#3	12	1.09	12.09

### 10.2. Diagnostic Specificity

The diagnostic 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%).

### 10.3. Diagnostic Sensitivity

The diagnostic 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%).

### 10.4. 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.

### 10.5. 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.

## 11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

## 12. PRECAUTIONS AND WARNINGS

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- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

### 12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

## 13. ORDERING INFORMATION

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Prod. No.:        HANG0670        Hantavirus IgG ELISA (96 Determinations)

# DEUTSCH

## 1. EINLEITUNG

Hantaviren sind Einzelstrang ss(-)RNA Viren der Bunyaviridae Familie. Die Infektion von Menschen findet durch Urin, Speichel oder Kontakt mit anderen Abfallprodukten von Nagetieren statt. Einige Hantaviren können ernsthafte Erkrankungen verursachen wie das Hämorrhagische Fieber mit Renalem Syndrom (HFRS) oder das Hantavirus-assoziierte pulmonale Syndrom, HPS.

Infektionen mit Hantaviren lassen sich fast vollständig auf den Kontakt mit Ausscheidungsprodukten von Nagetieren zurückführen, es wurden aber auch Mensch zu Mensch Übertragungen mit dem Andes Virus in Südamerika beschrieben.

Hantaviren haben eine Inkubationszeit von 2-4 Wochen bevor erste Symptome der Infektion auftreten. Die Symptome von HFRS können in 5 Phasen unterteilt werden:

- Febrile Phase: Die Symptome beinhalten Fieber, Schüttelfrost, Schweißausbrüche, Brechdurchfall, Unwohlsein, Kopfschmerzen, Übelkeit, abdominal und Rückenschmerzen, Atemprobleme wie sie auch durch gewöhnliche Influenzaviren hervorgerufen werden, als auch gastrointestinale Probleme. Die Symptome treten normalerweise 2-3 Wochen nach der Infektion für die Dauer von 3-7 Tagen auf.
- Hypotonische Phase: Diese tritt auf, wenn der Level an Thrombozyten fällt und Herzrasen sowie Hypoxämien auftreten. Diese Phase kann 2 Tage andauern.
- Oligurische Phase: Diese Phase dauert 3-7 Tage an und ist charakterisiert durch das Auftreten von Nierenversagen und Eiweißausscheidungen in Harn.
- Diuretische Phase: Diese Phase ist charakterisiert durch Harnmengen von 3-6 Litern pro Tag. Sie kann wenige Tage bis zu einigen Wochen andauern.
- Rekonvaleszente Phase: Hier findet die Erholung von der Krankheit statt und die Symptome gehen zurück.

Speziell von HFRS betroffene Regionen sind China, die koreanische Halbinsel, Russland (Hantaan, Puumala und Seoul Viren) sowie den Norden und Westen von Europa (Puumala und Dobrava Viren).

Spezies	Erkrankung	Symptome (z.B.)	Infektionsweg
Puumala Virus  Dobrava Virus  Hantaan Virus  Seoul Virus	Hämorrhagisches Fieber mit renalem Syndrom (HFRS)	Früh: plötzlich auftretende Symptome wie Kopf- und Rückenschmerzen, abdominale Schmerzen, Fieber, Schüttelfrost, Übelkeit und verschwommene Sicht.  Spät: Niedriger Blutdruck, akuter Schock, Gefäßdurchlässigkeitsstörung und akutes Nierenversagen.	Durch Einatmen von Stäuben, die Urin, Kot, Speichel oder Nestmaterial infizierter Nager enthalten (luftübertragene Transmission).  Ebenso durch direkten Kontakt mit diesen Materialien mit verletzter Haut oder Schleimhäuten.
Andes Virus Sin-Nombrevirus  (Neue Welt Stämme)	Hantavirus-induziertes- (kardio-) pulmonales Syndrom (HPS/ HCPS)	Früh: universell auftretende Symptome wie Schwindel, Fieber und Muskelschmerzen, v.a. der großen Muskulaturen von Oberschenkel, Hüfte, Rücken und manchmal Schultern. Ebenso können Kopfschmerzen, Benommenheit, Schüttelfrost, und abdominale Probleme wie Übelkeit, Erbrechen, Durchfall und Unterleibsschmerzen auftreten.  Spät: Husten und Kurzatmigkeit, Flüssigkeitsansammlung in den Lungen.	Durch Nagerbisse.  Eine Übertragung von Mensch zu Mensch kann nicht ausgeschlossen werden (v.a. bei den Neue Welt Stämmen).

Nachweis des Erregers bzw. der Infektion durch:

- PCR
- Serologie (z.B. ELISA)

## 2. VERWENDUNGSZWECK

Der Hantavirus IgG ELISA ist für den qualitativen Nachweis spezifischer IgG Antikörper gegen Hantavirus in humanem Serum oder Plasma (Citrat oder Heparin) bestimmt.

**NovaLisa®**

# Hantavirus IgM

**ELISA**

**CE**

**Only for in-vitro diagnostic use**

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Product Number:      HANM0670 (96 Determinations)

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## ENGLISH

### 1. INTRODUCTION

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 may cause serious diseases in humans, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS).

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.
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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).

Species	Disease	Symptoms (e.g.)	Transmission route
Puumala virus  Dobrava virus  Hantaan virus  Seoul virus	Hemorrhagic fever with renal syndrome (HFRS)	Initial: suddenly occurring symptoms like intense headache, back and abdominal pain, fever, chills, nausea, and blurred vision.  Late: low blood pressure, acute shock, vascular leakage, and acute kidney failure	After exposure to aerosolized urine, droppings, or saliva of infected rodents or their nests (airborne transmission).  Also by direct contact with these materials to broken skin or onto mucous membranes.
Andes virus  Sin-Nombre-virus  (New world strains)	Hantavirus pulmonary syndrome (HPS)	Early: universal symptoms include fatigue, fever and muscle aches, especially in the large muscle groups - thighs, hips, back, and sometimes shoulders. There may also be headaches, dizziness, chills, and abdominal problems, such as nausea, vomiting, diarrhea, and abdominal pain.  Late: coughing and shortness of breath, lungs fill with fluid.	Bites by infected rodents.  Human to human transmission can not be excluded (for New World strains).

The presence of pathogen or infection may be identified by:

- PCR
- Serology (e. g. ELISA)

### 2. INTENDED USE

The Hantavirus IgM ELISA is intended for the qualitative determination of IgM antibodies against Hantavirus in human serum or plasma (citrate or heparin).

### 3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

## 4. MATERIALS

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### 4.1. Reagents supplied

- **Hantavirus Coated Microplate (IgM):** 12 breakapart 8-well snap-off strips coated with recombinant Hantavirus antigens in resealable aluminium foil.
- **IgM Sample Diluent:** 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M); pH 7.2 ± 0.2; for washing the wells; white cap.
- **Hantavirus anti-IgM Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibody to human IgM; in phosphate buffer (10 mM); coloured red, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1%; ready to use; yellow cap; < 5% NMP.
- **Hantavirus IgM Positive Control:** 1 bottle containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Hantavirus IgM Cut-off Control:** 1 bottle containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Hantavirus IgM Negative Control:** 1 bottle containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

### 4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

### 4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes

## 5. STABILITY AND STORAGE

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Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

## 6. REAGENT PREPARATION

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It is very important to bring all reagents and samples to room temperature (20...25 °C) and mix them before starting the test run!

### 6.1. Coated Microplate

The break-apart snap-off strips are coated with recombinant Hantavirus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

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Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20...25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

### 6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

## 7. SAMPLE COLLECTION AND PREPARATION

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Use human serum or plasma (citrate or heparin) samples with this assay. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Heat inactivation of samples is not recommended.

### 7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10 µl sample and 1 ml IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

## 8. ASSAY PROCEDURE

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### 8.1. Test Preparation

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!  
Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

### 8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

**Measure the absorbance** of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

## 9. RESULTS

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### 9.1. Run Validation Criteria

In order for an assay to be considered valid, 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.

## 9.2. 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

### 9.2.1. Results in Units [NTU]

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

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

## 9.3. 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 <b>negative</b> .
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.

### 9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

## 10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

### 10.1. Precision

Intraassay	n	Mean (E)	Cv (%)
#1	24	0.649	4.11
#2	24	1.322	3.24
#3	24	1.064	4.29
Interassay	n	Mean (NTU)	Cv (%)
#1	12	23.49	12.94
#2	12	15.31	12.08
#3	12	0.73	12.86

### 10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 99.03% (95% confidence interval: 94.71% - 99.98%).

### 10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 96.3% (95% confidence interval: 89.56% - 99.23%).

### 10.4. 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.

### 10.5. 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.

## 11. LIMITATIONS OF THE PROCEDURE

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Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

## 12. PRECAUTIONS AND WARNINGS

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- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

### 12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

## 13. ORDERING INFORMATION

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Prod. No.:        HANM0670        Hantavirus IgM ELISA (96 Determinations)