

Multiplex Hantavirus-HFRS - Real Time RNA

50 /100 /150 tests (Ready to use kit)

MPX. Hanta-HFRS RNA Real Time PCR Kit is a screening assay for rapid and accurate detection of Dobrava Belgrade hantavirus, Seoul hantavirus, Hantaan hantavirus and Puumalahantavirus.

Principles of the test:

The Hantavirus-HFRS Multiplex qRT-PCR Kit of Bioingentech® The Hantavirus-HFRS Multiplex qRT-PCR Kit of Bioingentech® provides a rapid, reliable and cost effective molecular solution for the specific detection and differentiation of the four most clinically relevant hantaviruses responsible for Hemorrhagic Fever with Renal Syndrome (HFRS): Dobrava-Belgrade hantavirus (DOBV), Seoul hantavirus (SEOV), Hantaan hantavirus (HTNV) and Puumalahantavirus (PUUV). The assay provides clinical and public health laboratories with a powerful molecular tool for the rapid diagnosis of HFRS associated hantaviruses, transforming complex molecular testing into clear, accurate and actionable results.

The assay is organized into two multiplex Master Mixes to optimize analytical performance while maintaining broad diagnostic coverage. The first Master Mix simultaneously detects Dobrava-Belgrade hantavirus (DOBV) and Seoul hantavirus (SEOV), whereas the second Master Mix simultaneously detects Hantaan hantavirus (HTNV) and Puumala hantavirus (PUUV). An Internal Control (IC) is included in both reactions to verify nucleic acid extraction, reagent integrity and amplification performance, ensuring reliable interpretation of negative results.

The selected viral targets encompass the principal hantavirus species responsible for HFRS throughout Europe and Asia. Dobrava-Belgrade hantavirus is associated with severe forms of HFRS in southeastern Europe, Hantaan hantavirus causes the classical severe form of HFRS throughout East Asia, Seoul hantavirus has a worldwide distribution through infected rodent reservoirs and commonly produces moderate disease, while Puumala hantavirus is the predominant cause of nephropathia epidemica, the mildest clinical manifestation of HFRS in Northern and Central Europe.

The kit enables the specific detection of hantavirus RNA directly from clinical specimens collected from patients presenting with acute febrile illness compatible with HFRS. The assay is intended for use in clinical microbiology laboratories, hospital diagnostic centers, national reference laboratories and public health surveillance programs involved in the diagnosis and epidemiological monitoring of zoonotic viral infections.

By distributing the four viral targets into two optimized multiplex reactions, the assay minimizes reagent consumption and hands-on time while maintaining high analytical sensitivity and specificity. This workflow supports rapid laboratory diagnosis and reliable differentiation of the major HFRS-causing hantaviruses, facilitating patient management, outbreak investigations and molecular surveillance.

Master Mix DOBV & SEOV

DOBV in FAM and 3-quencher which can be detected through green channel.

SEOV in ROX and 3-quencher which can be detected through purple channel.

Master Mix HTNV & PUUV

HTNV in FAM and 3-quencher which can be detected through green channel.

PUUV in ROX and 3-quencher which can be detected through purple channel.

Principle and use:

This amplification kit has been manufactured by Bioingentech Ltd. Chile to detect Multiplex Hantavirus-HFRS in real time PCR. This is a possibility relative/absolute quantification or qualitative assay.

Real time PCR is based on fluorogenic dyes. Ct value between 0 – 36 should be taken positive. Value between 36-40 Ct should be taken as marginal positive. Ct above 40 must be considered as negative (**for more details see Table 5**). This kit needs RNA as a template which can be isolated from blood, serum, faeces, respiratory fluid, cerebrospinal fluid, digestive system, tissue, Heptopancreas, Gills, Pleopods, Cloacal, Egg Yolk, Milk, swabs, Lee, bacterial cultures, cell lines and others.

Table 1. Kit Components:

Reactions Tubes	50 test	100 test	150 test
Universal qPCR Master Mix	(2 unit)	(2 unit)	(2 unit)
Primer, Probes and Internal Control Mix	(2 unit)	(2 unit)	(2 unit)
One qRT-PCR Enzyme Mix	(2 unit)	(2 unit)	(2 unit)
Master Mix DOBV & SEOV	(2 unit)	(2 unit)	(2 unit)
Master Mix HTNV & PUUV	(2 unit)	(2 unit)	(2 unit)
MPX. Hanta-HFRS Positive Control	(2 unit)	(2 unit)	(2 unit)
MPX. Hanta-HFRS Negative Control	(2 unit)	(2 unit)	(2 unit)
PCR grade Water	(2 unit)	(2 unit)	(2 unit)
Exogenous RNA control	(2 unit)	(2 unit)	(2 unit)
Exogenous RNA control Primer and Probes Mix	(2 unit)	(2 unit)	(2 unit)

* Remember that all our OneVetqPCR-realtime™ MPX. Hanta-HFRS RNA. Real Time PCR Kits include reagents and procedures. Also we always can offer you a complete technical support at info@kitpcr.com

Table 2. Instrument Compatibility:

* ABI 7300	* Mastercycler® ep realplex
* ABI 7500FAST	* LightCycler 2.0 and 480
* ABI 7900	* Mx3000P QPCR System
* AB Step One	* Mx3005P QPCR System
* AB Step One Plus	* RotorGene 3000
* Agilent Mx3005P	* RotorGene 6000
* CFX96 & CFX384	* RotorGeneQ
* ExiCycler™ 96	* SLAN® Real-Time PCR
* iQ5 & MyiQ Cycler	* Smartcycles II
* Illumina Eco	* CFX Opus 96
* LightCycler Nano	* CFX Opus 384
* CFX Opus	* Quantstudio 3
* Bioer Quant gene 9600	* Quantstudio 5

Procedure:

Please read through the entire procedure before starting.

Before Starting

- Pulse-spin each tube in a centrifuge before opening.
- Homogenize the solutions for 5 seconds prior to pipetting.
- You must consider use different tips in order to avoid cross contamination.
- Use only sterile, RNAses, DNAses and pyrogens free tips.
- Add Exogenous RNA Control to the lysis/binding solution concentrate that is used for the RNA isolation. You must add 2 µL of undiluted Exogenous RNA Control (20,000 copies) per desirable isolation.

Step 1

Prepare a Master mix according to the reaction table 3.

Table 3. Reaction components for PCR

Tubes	Sample and Internal Control	Positive Control	Negative Control	Exogenous RNA Control
Universal qPCR Master Mix	7,5 µL	7,5 µL	7,5 µL	7,5 µL
Primer, Probes and Internal Control Mix	0,6 µL	0,6 µL	0,6 µL	
One qRT-PCR Enzyme Mix	0,5 µL			0,5 µL
Exogenous RNA control Primer and Probes Mix				0,6 µL
PCR grade Water	2,9 µL	3,4 µL	3,4 µL	2,9 µL
Exogenous RNA Control				3,5 µL
MPX. Hanta-HFRS Positive Control		3,5 µL		
MPX. Hanta-HFRS Negative Control			3,5 µL	
RNA Sample	3,5 µL			
Total Volume	15 µL	15 µL	15 µL	15 µL

Step 2

Place the tubes in a thermal cycler and perform One Step qPCR according to the program outlined in Table 4.

Table 4. Recommended PCR Cycling table

Cycles	Steps	Time	Temp. (°C)
1 Cycle	Reverse Transcription	20 min	45 °C
1 Cycle	Denaturation Step	2 min	95 °C
40 Cycles	Denaturation	10 seg	95 °C
	Annealing	1 min	60 °C
	Hold	----	4 °C

Note 1: Collect data in 60 °C

Note 2: Hold Step is optional.

Interpretation of the test

1) Qualitative analysis:

Ct (Threshold cycle) value of each sample can be read as follows.

Table 5. Ct value result

Ct value	Result
0 - 36	Positive
36 - 40	Marginal Positive
> 40	Negative

1.1.-Ct values over 40 are considered Negative. If the Ct value is in the 0 - 36 range, it must be considered as Positive. This is depending of the sample initial concentration use d for each reaction. Note that the sample real concentration could be altered by the sample purity when it is quantified.

1.2.- For more technical information you must request the **QUALITY CONTROL** for each kits. Also you can request more information writing to our email info@kitpcr.com

2) Quantitative analysis:

Table 6. Preparation of standard curve dilution series. MPX. Hanta-HFRS positive control:

Average Positive Control Concentration	
MPX. Hanta-HFRS	See Quality Control

Standard curve	Preparation series a fresh dilution	Concentration	Copy Number
Tube N°1:	2uL MPX. Hanta Positive Control (2 ng/μL) + 18 μL de PCR grade Water	See quality control	See quality control
Tube N°2:	2uL Tube N°1 + 18 μL de PCR grade Water	See quality control	See quality control
Tube N°3:	2uL Tube N°2 + 18 μL de PCR grade Water	See quality control	See quality control
Tube N°4:	2uL Tube N°3 + 18 μL de PCR grade Water	See quality control	See quality control
Tube N°5:	2uL Tube N°4 + 18 μL de PCR grade Water	See quality control	See quality control
Tube N°6:	2uL Tube N°5 + 18 μL de PCR grade Water	See quality control	See quality control
Tube N°7:	2uL Tube N°6 + 18 μL de PCR grade Water	See quality control	See quality control

- 2.1. Important Note: Don't forget Homogenize the tubes.
 2.2. We will send a Quality Control report for each purchase.
 2.3. If you want to obtain less DNA copies you must include a new dilution tube (**Tube N°8**).
 2.4. Final DNA copy number will depend of the DNA concentration (**you can see it in Quality Control Report**).

Table 7. Standard curve set up

For each of the tubes (tube 1 to tube 7), take 3,5 μl of the final 15 μl mixture.

	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F	Tube G
Universal qPCR Master Mix	7,5 μL	7,5 μL	7,5 μL	7,5 μL	7,5 μL	7,5 μL	7,5 μL
Primer, Probes and Internal Control Mix	0,6 μL	0,6 μL	0,6 μL	0,6 μL	0,6 μL	0,6 μL	0,6 μL
PCR grade Water	3,4 μL	3,4 μL	3,4 μL	3,4 μL	3,4 μL	3,4 μL	3,4 μL
Tube N° 1 (Positive Control)	3,5 μL						
Tube N° 2		3,5 μL					
Tube N° 3			3,5 μL				
Tube N° 4				3,5 μL			
Tube N° 5					3,5 μL		
Tube N° 6						3,5 μL	
Tube N° 7							3,5 μL
Total Volume	15 μL	15 μL	15 μL	15 μL	15 μL	15 μL	15 μL

Take table N°6

- 2.5.- Assess the Ct value when the amplification curve of Standard tubes 1, 2, 3, 4, 5, 6, 7 passes the threshold line. Four tubes are sufficient for a standard curve (tubes 1 to 4).
 2.6.- Calculate the quantitative value in order to compare the Ct value of the unknown samples with the standard curve.
 2.7.- When visualizing the result for the Positive Control in the Real Time thermal cycler, there will be only one amplification curve for the target gene. It will not have an amplification curve for the Internal Control.

3) Test validation:

- 3.1.- Each Ct value standard should be as follows. Standard 1 < Standard 2 < Standard 3 < Standard 4 < Standard 5 < Standard 6 < Standard 7.
 3.2.- R-value of standard curve should be 0.900 - 0.999. R-value represent how well the experimental data fit the regression line. A significant difference in observed Ct values between replicates will lower the R-value.
 3.3.- The standard curve slope result should be all negative.
 3.4.- The desired amplification efficiencies vary from 90% to 110%. The theoretical maximum of 100% indicates that the polymerase enzyme is functioning at its maximum capacity. Low reaction efficiencies may be caused by poor primer design or by suboptimal reaction conditions. Reaction efficiencies >110 may indicate pipetting error in your serial dilutions or coamplification of non specific products, such as primer-dimers.

Visual explanation FAQ:

1.-Positive control:

The Positive control assay uses a FAM and ROX dyes and should be detected through the Green and Purple channels of your real time PCR instrument. For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of Multiplex Hantavirus-HFRS copy number / Ct value.

Alternatively, the positive control can be used at a single dilution Multiplex Hanta virus on where full quantitative analysis of the sample is not required. Each time the kit is used, at least one positive control reaction must be included in the run. Particularly, due to amount of this reagent, you should run a positive control for each 12 samples. A positive result indicates that the primer and probes for detecting the target Multiplex Hantavirus-HFRS gene worked properly in that particular experimental scenario. If a negative result is obtained the test results should be invalid and must be repeated (**see Table 11**). Sealing all other samples and negative controls before pipetting the positive control into the positive control well tube.

2.-Internal Control:

The internal control is included in Primer, Probes and Internal Control Mix along to the target pathogen detection. In order to interpreted results, read the yellow channel. The internal control assay uses a HEX dye and should be detected through the Yellow channel of your real time PCR instrument and gives a Ct value of 28 (+/-5) depending on the level of sample dilution and concentration. A positive result through the Yellow channel the before indicates that PCR conditions are suitable for detection of the target pathogen gene. If a negative result is obtained through the Yellow channel the results should be analyzed by combination of result, follow the **Table 11 data**.

3.-Negative control:

To confirm absence of contamination a negative control reaction should be included every time the kit is used. Particularly, due to amount of this reagent, you should run a negative control for each 12 samples. In this instance the Multiplex Hantavirus-HFRS Negative Control should be used in place of template. A negative result indicates that the reagents have not become contaminated. If a positive result and Ct value less than 36 is obtained, the results should be analyzed and check if a correct amplification curve was obtained. When you obtain a clear amplification curve you should consider repeat your assay due to probably the sample was contaminated (see Table 11).

* Remember: We recommend running a Positive control and Negative control for each 12 samples. For reaction mix you must use Universal qPCR Master Mix.

4.-Internal RNA control:

An exogenous source of RNA template is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Amplification of this control cDNA does not interfere with detection of the target cDNA even when present at low copy number. Previous of PCR setup you must add 2µl (20.000 copies) of undiluted Exogenous RNA Control to the lysis/binding solution concentrate that is used for the RNA isolation.

The Internal control is detected through the Yellow channel and gives a Ct value up to 30 depending on the level of sample dilution. Successful co-purification and qPCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration. To confirm this, Internal RNA control should be included every time the kit is used. **Particularly, due to amount of this reagent, we recommend including and running one Internal RNA control for each 12 samples.**

* Exogenous RNA control is just referential and its negative result should not be interpreted as a failed assay when the sample and internal control give a positive result (see Table 11).

Table 8. Fluorogenic probes, Channels and Dyes

* In order to setup your software to read the probes, (see Table 8 and 9) and run the corresponding channel:

Channel	Source	Detector	Dyes
Green	470(Nm)	520(Nm)	FAM, Sybr green1, Fluorescein, Eva green, Alexa fluor 488
Yellow	530(Nm)	550(Nm)	Joe, Vic, HEX Tet, Cal Fluorgold 540, YaKima Yellow
Purple	585(Nm)	610(Nm)	ROX, Cal Fluor Red 610, Cy3.5, Texas Red, Alexa Fluor 568
Red	625(Nm)	660(Nm)	Cy5, Quasar 670, Lightcycler, Red 640, Alexa Fluor 633, Aeon ZW™.
Crimson	680(Nm)	710(Nm)	Quasar 705, Lightcycler Red 705, Alexa Fluor 680

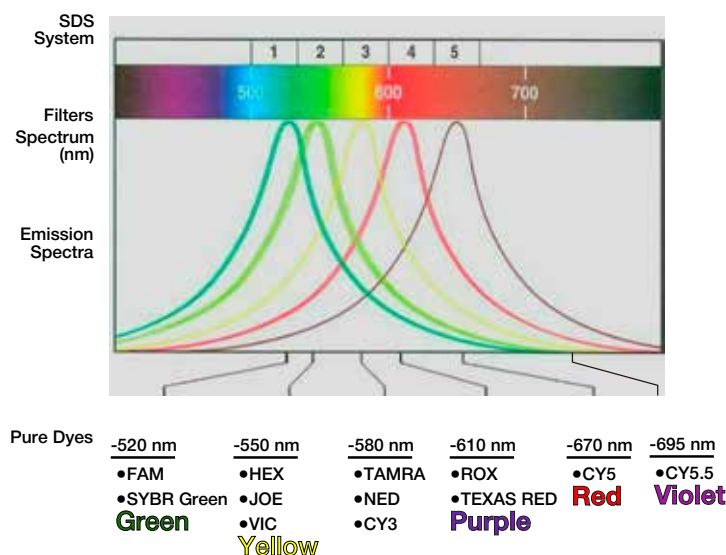


Table 9. Similarity of our fluorophores with HEX, FAM and ROX dyes.

	Channel	Dyes
1.- Positive Control	Green	FAM
2.- Positive Control	Purple	ROX
3.- Internal Control	Yellow	HEX
4.- Sample Target	Green	FAM
5.- Sample Target	Purple	ROX
6.- Exogenous RNA control	Yellow	HEX

Important Note:

Request a Certificate of Analysis when you purchase one of our PCR Kits.

Is important mentioned that we can develop special request for other pathogens or multiplex detection according client's specifications.

We strongly recommend don't use or combine our products with reagents from another kits or unknown provenance. We can't assure good result if incompatibility problems occur.

Temperature

All our reagents are made through protein engineering and are stable at room temperature, the label temperature is just a recommendation after the product is open.

Table 10. Store Temperature Kits

	Store temperature	The label temperature
	Shipping temperature	At room temperature

Table 11. Interpretation of Results

Sample	I.C	N.C	P.C	eRNA Control	Result
+	+	-	+	+	POSITIVE
+	-	-	+	+	POSITIVE
+	+	-	+	-	POSITIVE
+	-	-	+	-	POSITIVE
+	+	+	+	+	Check Ct and Consider repeat assay*
+	-	+	+	+	Check Ct and Consider repeat assay*
+	+	+	+	-	Check Ct and Consider repeat assay*
+	+	-	-	-	Check Ct and Consider repeat assay*
+	+	+	-	-	Check Ct and Consider repeat assay*
+	+	-	-	+	Exp. Fail
+	+	+	-	+	Exp. Fail
+	-	-	-	-	Exp. Fail
+	-	-	-	+	Exp. Fail
+	-	+	-	-	Exp. Fail
-	+/-	+/-	+/-	+/-	NEGATIVE

* Sometimes amplification curves for Negative or Internal control with Ct < 30 can be seen, however it might not be a Positive result. If the amplification curve is not sigmoid, you should consider it as a Negative result.

Table 12. Other Products

Products
OneVetqPCR-realtime™ MPX. Hanta-HFRS Multiplex Hantavirus-HFRS Real Time 50 tests (Ready to use kit) / Cat. No: MulOneq-H874-50R
OneVetqPCR-realtime™ MPX. Hanta-HFRS Multiplex Hantavirus-HFRS Real Time 100 tests (Ready to use kit) / Cat. No: MulOneq-H874-100R
OneVetqPCR-realtime™ MPX. Hanta-HFRS Multiplex Hantavirus-HFRS Real Time 150 tests (Ready to use kit) / Cat. No: MulOneq-H874-150R

Table 13. Products Specifications

Technology	5' nuclease probe based real time PCR assay
Type of nucleic acid Kit	RNA
Kit storage	Shipped at room temperature, the label temperature is just a recommendation after the product is open.
Detection Limit	See Quality Control file. Request it!
Sensitivity & Specify	Ct value between 0 – 36 should be taken positive. Value between 36-40 Ct should be taken as marginal positive. Ct values above 40 must be considered as negative.
Controls included	Internal control, Positive control and Negative control included.
Channels	FAM Green channel detect pathogen aplicons. ROX Purple channel detect pathogen aplicons. HEX Yellow channel detect internal control amplicons. FAM Green channel detect Positive Control. ROX Purple channel detect Positive Control. HEX Yellow channel detect Exogenous RNA control.