

Hantavirus RNA Detection Kit

Real-Time RT-PCR Kit for the Detection of Hantavirus (N gene & RdRp)

Catalog No.: QP2200-01 (100-test) | QP2200-02 (500-test)

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Ordering Information

#	Component	Description	100-Test Kit	500-Test Kit
1	HTV PCR Mix	Ready-to-use multiplex RT-PCR mix (primers, probes, dNTPs, Taq, RT enzyme, UDGase, buffer)	1.6 mL/tube × 1	1.6 mL/tube × 5
2	Positive Control	Synthetic control containing all target sequences (N gene, RdRp, and RNase P)	50 µL/tube × 1	250 µL/tube × 1
3	Negative Control	Nuclease-free water	50 µL/tube × 1	250 µL/tube × 1

1. Intended Use

The Biomiga Hantavirus RNA Detection Kit (QP2200) is a multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) assay intended for the qualitative detection and differentiation of Hantavirus RNA in nucleic acid extracts derived from respiratory specimens (nasopharyngeal swabs, bronchoalveolar lavage) and whole blood. The kit simultaneously amplifies two independent genomic targets: the nucleocapsid protein gene (N gene, S segment) detected in the FAM channel and the RNA-dependent RNA polymerase gene (RdRp, L segment) detected in the ROX channel. Human RNase P (VIC channel) serves as an internal control to verify specimen adequacy and extraction efficiency.

This product is FOR RESEARCH USE ONLY (RUO). It is not intended for use in diagnostic procedures.

2. Summary and Background

Hantaviruses (family Hantaviridae, genus Orthohantavirus) are negative-sense, single-stranded RNA viruses with a tripartite genome comprising the Small (S), Medium (M), and Large (L) segments. The S segment encodes the nucleocapsid (N) protein, the M segment encodes the glycoproteins Gn and Gc, and the L segment encodes the RNA-dependent RNA polymerase (RdRp). Hantaviruses are maintained in nature in rodent reservoir hosts and are transmitted to humans primarily through inhalation of aerosolized infectious excreta (urine, feces, saliva) or, less frequently, through direct contact with infected animals.

Hantavirus infections in humans manifest as two distinct clinical syndromes depending on the infecting species and geographic region. Hemorrhagic Fever with Renal Syndrome (HFRS) is caused predominantly by Old World hantaviruses (Hantaan virus, Seoul virus, Puumala virus, Dobrava virus) and is endemic across Eurasia, with a case fatality rate ranging from less than 1% (Puumala) to 15% (Hantaan and Dobrava). Hantavirus Cardiopulmonary Syndrome (HCPS), also known as Hantavirus Pulmonary Syndrome (HPS), is caused by New World hantaviruses including Sin Nombre virus, Andes virus, and related strains predominantly circulating in the Americas; HCPS carries a significantly higher case fatality rate of 30 to 40%.

The global distribution of hantavirus disease is expanding, with recognized outbreaks reported across Europe, Asia, North and South America, and emerging detections in Africa. Climate change-driven shifts in rodent population dynamics, increased human encroachment into endemic habitats, and international travel have collectively heightened the risk of hantavirus exposure in both endemic and non-endemic regions. Seoul virus, carried by the common brown rat (*Rattus norvegicus*), represents the only hantavirus with global distribution due to worldwide rodent trade and urban infestation.

Early clinical presentation of hantavirus infection is nonspecific, resembling influenza-like illness with fever, myalgia, headache, and fatigue, which frequently delays diagnosis. The prodromal phase is followed by rapid progression to severe pulmonary edema (HCPS) or acute kidney injury with thrombocytopenia (HFRS). Given the high mortality associated with delayed recognition and supportive care requirements, rapid and sensitive molecular diagnostics are critical for patient management and outbreak response.

Molecular detection targeting highly conserved genomic regions offers the most sensitive and specific approach for hantavirus diagnosis, particularly during the early viremic phase before seroconversion. Biomiga has developed this real-time RT-PCR

assay targeting two independent conserved regions: the N gene on the S segment and the RdRp on the L segment. Dual-target design provides redundant detection, reduces the risk of false negatives due to sequence variation in individual targets, and facilitates differentiation of true positives from background signals. Assay design incorporated analysis of prevailing strains including Sin Nombre virus, Andes virus, Hantaan virus, Seoul virus, Puumala virus, and Dobrava virus, with primers and probes designed against regions of highest inter-strain conservation.

The assay achieves an analytical sensitivity of 1 copy per PCR reaction, corresponding to a Limit of Detection (LoD) of 200 copies/mL in the original specimen, established through probit regression analysis across replicate testing at serial dilutions. RNase P detection in the VIC channel serves as an internal control for nucleic acid extraction efficiency, specimen quality, and PCR inhibition, ensuring the validity of negative results.

3. Panel Composition

Fluorescence Channel	Target	Function
FAM	N gene (S segment)	Hantavirus detection
ROX	RdRp (L segment)	Hantavirus detection
VIC	RNase P	Internal Control (extraction & inhibition)

4. Reagents Provided

Each kit contains the following components:

- PCR Mix (1.6 mL/tube): Ready-to-use multiplex RT-PCR master mix containing optimized concentrations of sequence-specific primers and dual-labeled TaqMan probes for N gene (FAM), RdRp (ROX), and RNase P (VIC); dNTPs with dUTP substitution; thermostable DNA polymerase with integrated reverse transcriptase activity; UDGase (uracil-DNA glycosylase) for carry-over contamination control; reaction buffer with MgCl₂; and stabilizing additives. No user additions are required beyond template.
- Positive Control (50 µL or 250 µL/tube): Synthetic RNA transcript containing all target sequences (N gene, RdRp, RNase P). Store at -70 °C or below. Avoid freeze-thaw cycles.
- Negative Control (50 µL or 250 µL/tube): Nuclease-free water; serves as a no-template control (NTC).

5. Materials Required But Not Provided

- Compatible nucleic acid extraction kit (e.g., Biomiga ER Series or equivalent RNA extraction system)
- Compatible automated nucleic acid extractor (e.g., Biomiga MyPureMini 16/32, MyPure 96, Thermo KingFisher Flex, or Allsheng AutoPure 96) or manual extraction method
- Compatible real-time PCR thermocycler (see Section 11)
- Optical 96-well PCR plates or tubes compatible with thermocycler
- Optical adhesive sealing film
- Vortex mixer, microcentrifuge, and bench-top centrifuge
- Calibrated micropipettes and aerosol-barrier (filter) tips
- Personal protective equipment: gloves, lab coat, eye protection
- RNase-free microcentrifuge tubes

6. Specimen Collection, Transport, and Storage

Specimen collection should follow current biosafety guidelines. Hantavirus is classified as a BSL-3 pathogen; handle all primary specimens in an appropriate biosafety cabinet. Appropriate specimen types include:

- Nasopharyngeal swabs (NPS): Collect in viral transport medium (VTM). Transport at 2-8 °C; process within 72 hours.
- Bronchoalveolar lavage (BAL): Collect into sterile container. Transport on wet ice; process within 24 hours.
- EDTA whole blood: Collect in EDTA (purple-top) tube. Transport at 2-8 °C; process within 48 hours.

If immediate processing is not possible, aliquot specimens and store at -70 °C or below. Avoid repeated freeze-thaw cycles (maximum 3 cycles). Do not use heat-inactivated specimens without validation.

7. Nucleic Acid Extraction

Extract total RNA/DNA from specimens using a validated nucleic acid extraction method compatible with respiratory specimens and whole blood. Biomiga ER-series extraction kits used with Biomiga MyPureMini 16/32 or MyPure 96 automated extractors are recommended.

General guidance:

- Input volume: 200 µL specimen recommended (adjust per extraction kit protocol).
- Elute in 50-100 µL nuclease-free water or elution buffer.
- Use the extracted nucleic acid immediately or store at -70 °C or below.
- Perform extraction in a designated pre-PCR area to minimize contamination risk.
- Include an extraction negative control (nuclease-free water) in each extraction batch.

8. Reaction Setup

Prepare reactions in a designated pre-PCR area, physically separated from post-amplification areas. Use aerosol-barrier tips at all times.

Component	Volume per Reaction
PCR Mix	15 µL
Negative Control / Positive Control / Extracted RNA from specimen	5 µL
Total Reaction Volume	20 µL

1. Thaw the PCR Mix on ice; vortex briefly and centrifuge to collect contents.
2. Dispense 15 µL of PCR Mix into each well or tube.
3. Add 5 µL of extracted RNA, Positive Control, or Negative Control to the respective wells.
4. Seal the plate with optical adhesive film; centrifuge at 1,000 x g for 1 minute to remove air bubbles.
5. Load plate into thermocycler and initiate run immediately.

9. PCR Cycling Protocol

Cycles	Step	Duration	Temperature
1	Contamination Removal (UDG Activation)	2 min	37 °C
1	Reverse Transcription	10 min	50 °C
1	Initial Denaturation	2 min	95 °C
40	Denaturation	5 sec	95 °C
	Annealing / Extension	20 sec	60 °C

The PCR Mix contains UDGase to enzymatically degrade uracil-containing DNA carry-over amplicons at 37 °C prior to reverse transcription. The 50 °C reverse transcription step converts viral RNA to cDNA. Standard two-step PCR cycling at 95/60 °C is then employed for amplification and detection.

10. Result Interpretation

Set the fluorescence threshold manually or allow the thermocycler software to set it automatically, ensuring the threshold intersects the exponential amplification phase above background noise. Evaluate results as follows:

N gene (FAM)	RdRp (ROX)	RNase P (VIC)	Interpretation
Ct ≤37, exponential	Ct ≤37, exponential	Any	HANTAVIRUS DETECTED - Both targets confirmed; report as Positive.
Ct ≤37, exponential	Not detected	Detected	HANTAVIRUS DETECTED (N gene only) - Repeat testing recommended; may reflect strain variant with RdRp divergence.
Not detected	Ct ≤37, exponential	Detected	HANTAVIRUS DETECTED (RdRp only) - Repeat testing recommended; may reflect strain variant.
Not detected	Not detected	Detected	HANTAVIRUS NOT DETECTED - Specimen is adequate; no Hantavirus RNA detected above the LoD.
Not detected	Not detected	Not detected	INVALID - RNase P internal control failed. Repeat extraction and/or dilute specimen 1:5 to address inhibition.

A result is considered POSITIVE when Ct ≤ 37 and the amplification curve exhibits an exponential (sigmoidal) shape. Ct values between 35 and 37 with weak sigmoidal curves should be interpreted with caution and may warrant repeat testing. Results should be interpreted in the context of epidemiological exposure history, clinical presentation, and other laboratory findings.

11. Compatible Real-Time PCR Thermocyclers

This kit has been verified for compatibility with the following thermocycler platforms:

Manufacturer	Model
Applied Biosystems	7500 Real-Time PCR System
Applied Biosystems	7300 Plus Real-Time PCR System
Thermo Fisher Scientific	QuantStudio 5
Thermo Fisher Scientific	QuantStudio 6 / 7
Thermo Fisher Scientific	QuantStudio 12K Flex
Bio-Rad	CFX96 Real-Time System

Configure the instrument as follows: FAM channel for N gene; ROX channel for RdRp; VIC channel for RNase P. On Applied Biosystems instruments using ROX as a passive reference dye, use a separate ROX-calibrated instrument for the ROX target channel; consult Biomiga Technical Support for instrument-specific configuration guidance.

12. Quality Control

Each run must include the following controls:

- Positive Control: Both N gene (FAM) and RdRp (ROX) must amplify with Ct ≤ 37 and exponential curves. RNase P (VIC) amplification is not expected in the synthetic RNA positive control.
- Negative Control (NTC): No amplification in FAM, ROX, or VIC channels. Any Ct ≤ 37 in the NTC indicates contamination; invalidate the run and decontaminate workspace.

Results are invalid if control acceptance criteria are not met. Do not report specimen results from an invalid run.

13. Storage and Stability

- PCR Mix: Store at -20 °C, protected from light. Stable until the expiration date printed on the label. Avoid repeated freeze-thaw cycles (maximum 5 cycles).
- Positive Control: Store at -70 °C or below. Aliquot upon first use to avoid freeze-thaw cycling.
- Negative Control: Store at -20 °C or room temperature (nuclease-free water). Stable for 12 months.
- Do not use reagents beyond their expiration date.

14. Limitations

- This kit is for research use only and is not intended or validated for use in diagnostic procedures.
- Performance has been established for the specimen types described herein. Use with other specimen types requires independent validation.
- A negative result does not exclude Hantavirus infection if collected outside the optimal viremic window or below the assay LoD.
- Discordant single-target results should prompt repeat testing and collection of a fresh specimen.
- This assay does not differentiate between individual Hantavirus species or strains.
- The presence of PCR inhibitors in specimens may cause false-negative results. The RNase P internal control monitors for inhibition.
- Concurrent infection with multiple pathogens will not interfere with detection of Hantavirus targets in this assay.

15. Warnings and Precautions

- Hantavirus is classified as a BSL-3 pathogen. All primary specimens must be handled in a certified biosafety cabinet following institutional biosafety policies and applicable regulations.
- This product contains chemical reagents. Refer to the Safety Data Sheet (SDS) for handling precautions.
- Do not mix reagents from different lot numbers within a single run without independent validation.
- Dedicated pre- and post-amplification areas and equipment are required to minimize carry-over contamination risk.
- Wear appropriate personal protective equipment (gloves, lab coat, eye protection) when handling specimens and reagents.
- Dispose of all materials in accordance with local, state, and federal biohazard waste regulations.
- Do not use kits beyond the expiration date or if the packaging is damaged.

16. Troubleshooting

Problem	Possible Cause	Corrective Action
No amplification in Positive Control	Reagent degradation; pipetting error	Check storage conditions; repeat with fresh reagents
RNase P (VIC) not detected in specimen	Extraction failure or PCR inhibition	Repeat extraction; dilute specimen 1:5 if inhibition suspected
Amplification in Negative Control	Contamination	Decontaminate workspace; invalidate run; repeat
High Ct values (Ct 35-37) with weak curve	Low viral load near LoD; inhibition	Report as detected; confirm with orthogonal method if clinically necessary
Discordant N gene / RdRp results	Strain variant; specimen quality issue	Repeat test; collect fresh specimen; contact Biomiga Technical Support

17. Performance Characteristics

Analytical Sensitivity (Limit of Detection):

- N gene (FAM): 1 copy per PCR reaction; 200 copies/mL in the original specimen.
- RdRp (ROX): 1 copy per PCR reaction; 200 copies/mL in the original specimen.
- LoD was determined by probit regression analysis using serial 3-fold dilutions of quantified synthetic RNA standards across 20 replicates per dilution level.

Analytical Specificity:

- In silico cross-reactivity analysis was performed against all sequences in the NCBI nucleotide database. No significant cross-reactivity (>80% homology) was identified with common respiratory pathogens including SARS-CoV-2, Influenza A/B, RSV, or other members of the Bunyaviridae family.

- Prevailing Hantavirus strains incorporated in assay design: Sin Nombre virus, Andes virus, Hantaan virus, Seoul virus, Puumala virus, and Dobrava-Belgrade virus.

Inclusivity:

- Primers and probes are designed against conserved regions verified to detect all included strains. Minor sequence variants may result in reduced sensitivity for novel or highly diverged strains.

18. Manufacturer Information

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20. Revision History

Revision	Date	Author	Description of Changes
Rev A	2026-06-01	Biomiga R&D	Initial release

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