# Hantavirus RNA Detection Kit

Real-Time RT-PCR Kit for the Detection of Hantavirus

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 | Hantavirus RT-PCR Mix | ready-to-use multiplex RT-PCR mix | 1.6 mL/tube × 1 | 1.6 mL/tube × 5 |
| 2 | Positive Control | positive control for targets and internal control | 50 µL/tube × 1 | 250 µL/tube × 1 |
| 3 | Negative Control | negative control for targets and internal control | 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 of Hantavirus RNA in nucleic acid extracts derived from respiratory specimens (nasopharyngeal swabs, bronchoalveolar lavage) and whole blood. The kit is designed to simultaneously detect two independent viral genomic targets: the nucleocapsid protein (N) gene on the S segment and the RNA-dependent RNA polymerase (RdRp) gene on the L segment. Amplification of the N gene is monitored in the ROX channel, while amplification of the RdRp gene is monitored in the FAM channel. Human RNase P is detected in the VIC channel and functions as an internal control to confirm specimen adequacy and the performance of the nucleic acid extraction process. **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, in particular the South American Andes virus lineages that caused the recent Hantavirus outbreak. *In silico* exclusivity analysis confirms no cross reactivity with the following viruses: SARS-CoV-2, Influenza A virus, Influenza B virus, Respiratory Syncytial Virus (RSV), Bunyaviridae, Yellow Fever Virus, Dengue Virus, Japanese Encephalitis Virus, West Nile Virus, St. Louis Encephalitis Virus, Ebola Virus, Sindbis Virus, Venezuelan Equine Encephalitis Virus, Crimean-Congo Hemorrhagic Fever Virus, Rift Valley Fever Virus, Severe Fever and Thrombocytopenia Syndrome Virus, Toscana Virus, Hendra Virus, Nipah Virus, Lassa Virus.

The assay demonstrated an analytical sensitivity of 1 copy per PCR reaction using serially diluted target material, corresponding to a Limit of Detection (LoD) of 200 copies/mL in the original specimen. 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 |
|---|---|---|
| ROX | N gene (S segment) | Hantavirus detection |
| FAM | RdRp (L segment) | Hantavirus detection |
| VIC | RNase P | Internal Control (extraction & inhibition) |

## 4. Reagents Provided

Each kit contains the following components:

- RT-PCR Mix (1.6 mL/tube): Ready-to-use multiplex RT-PCR master. No user additions are required beyond template.
- Positive Control (50 µL or 250 µL/tube): Synthetic construct containing all target sequences (N gene, RdRp, RNase P). Store at -20 °C. Avoid freeze-thaw cycles.
- Negative Control (50 µL or 250 µL/tube): 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 |
|---|---|
| RT-PCR Mix | 15 µL |
| Negative Control / Positive Control / Extracted RNA from specimen | 5 µL |
| **Total Reaction Volume** | **20 µL** |

1. Thaw the RT-PCR Mix on ice; vortex briefly and centrifuge to collect contents.
2. Dispense 15 µL of RT-PCR Mix into each well or tube.
3. Add 5 µL of extracted RNA, Positive Control, or Negative Control to the respective wells or tubes.
4. Seal the plate with optical clear adhesive film if 96-well PCR plates were used; 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 RT-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 (ROX) | RdRp (FAM) | 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: ROX channel for N gene; FAM 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.

## 12. Quality Control

Each run must include the following controls:

- Positive Control: Both N gene (ROX) and RdRp (FAM) must amplify with Ct ≤ 37 and exponential curves. RNase P (VIC) amplification is expected in the synthetic positive control.
- Negative Control (NTC): No amplification in FAM, ROX, or VIC channels. Any Ct ≤ 37 in the NTC indicates contamination; invalidates 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

- RT-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). Aliquot upon first use to avoid freeze-thaw cycling.
- Positive Control: Store at -20 °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 |

## 17. Performance Characteristics

Analytical Sensitivity (Limit of Detection):

- N gene (ROX): 1 copy per PCR reaction; 200 copies/mL in the original specimen.
- RdRp (FAM): 1 copy per PCR reaction; 200 copies/mL in the original specimen.
- LoD was determined by serial 10-fold dilutions of quantified standards.

Analytical Specificity:

- In silico cross-reactivity analysis, 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.
- No cross-reactivity were found against the following pathogens during in silico analysis: SARS-CoV-2, Influenza A, Influenza B, RSV, Bunyaviridae, Yellow Fever Virus, Dengue Virus, Japanese Encephalitis Virus, West Nile Virus, St. Louis Encephalitis Virus, Ebola Virus, Sindbis Virus, Venezuelan Equine Encephalitis Virus, Crimean-Congo

Hemorrhagic Fever Virus, Rift Valley Fever Virus, Severe Fever and Thrombocytopenia Syndrome Virus, Toscana Virus, Hendra Virus, Nipah Virus, Sin Nombre Virus, Lassa Virus.

Inclusivity:

- Primers and probes are designed against conserved regions of prevailing Hantavirus 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 | 2026-06-24 | Biomiga R&D | Initial release |