# **food**proof® Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit

## Revision A, September 2023

Real-time RT-PCR kit for the qualitative detection of norovirus genogroup I and II and hepatitis A virus RNA using real-time instruments

## Product No. KIT230056

**Kit for 64 reactions for a maximum of 62 samples**

**Store the kit at -15 to -25 °C**

For food testing purposes.

**FOR *IN VITRO* USE ONLY**

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## 1. Product Overview

### 1.1 Number of Tests

The kit is designed for 64 reactions [Master Mix (vial 1, yellow cap)] with a final reaction volume of 25 µL each. Up to 62 samples plus one positive control [Control Template (vial 4, purple cap)] and one negative control [Negative Control (vial 5, orange cap)] reaction can be analyzed per run.

#### *1.1.1 Process Control*

The kit contains a solution of Process Control (Bacteriophage MS2). The Process Control can be applied to the sample before starting the virus concentration procedure.

### 1.2 Storage and Stability

- Store the kit at -15 to -25 °C until the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following table of Kit Contents

| Vial | Label | Contents / Function / Storage |
|---|---|---|
| 1 Yellow cap | foodproof® Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit Master Mix | • 2 x 500 µL<br>• Ready-to-use primer and hydrolysis probe mix specific for norovirus GI and GII, hepatitis A virus, and the Process Control / Internal Amplification Control<br>• For amplification and detection of norovirus and hepatitis A virus RNA sequences.<br>• Store at -15 to -25 °C.<br>• **Avoid repeated freezing and thawing!**<br>• **Protect from light!** |
| 2 Red cap | foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit Enzyme Solution | • 2 x 40 µL<br>• Contains Reverse Transcriptase and a yellow dye for better visualization.<br>• Store at -15 to -25 °C.<br>• **Avoid repeated freezing and thawing!**<br>• **Protect from light!** |
| 3 White cap | foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit Process Control | • 3 x 250 µL<br>• Contains a stabilized solution of bacteriophage MS2.<br>• Added to the samples before starting the virus concentration procedure.<br>• For use as preparation / internal amplification control.<br>• Store at -15 to -25 °C.<br>• Only thaw RNA on ice or in 4 °C cooling block!<br>• **Avoid repeated freezing and thawing!** |
| 4 Purple cap | foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit Control Template | • 1 x 140 µL<br>• Contains a stabilized solution of DNA specific for norovirus GI and GII, hepatitis A virus, and the Process Control.<br>• For use as positive control with internal amplification control.<br>• Store at -15 to -25 °C.<br>• **Avoid repeated freezing and thawing!** |

| Vial | Label | Contents / Function / Storage |
| --- | --- | --- |
| 5<br>Orange cap | foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit<br>Negative Control | • 1 x 140 µL<br>• Contains a stabilized solution of DNA of the Process Control.<br>• For use as negative control with internal amplification control.<br>• Store at -15 to -25 °C.<br>• **Avoid repeated freezing and thawing!** |
| 6<br>Colorless cap | foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit<br>$H_2O$ PCR-grade | • 3 x 1 mL<br>• Nuclease-free, PCR-grade $H_2O$.<br>• For use as dilution reagent.<br>• After first thawing, store at 2 to 8 °C. |

### 1.3 Product Description/Applicability

The foodproof® Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit is a one-step real-time reverse transcriptase PCR for the simultaneous, qualitative detection of norovirus genogroups I (GI) and II (GII), hepatitis A virus (genotype I, II and III), and a Process Control / internal amplification control for comprehensive and fast interpretation of results. The kit provides primers and hydrolysis probes (for sequence-specific detection), convenient premixed reagents, and a control template for reliable interpretations of results. The foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit is based on primers, probes, and methods which are mentioned in the ISO/TS 15216 [4] and § 64 LFGB.
Norovirus and hepatitis A virus are highly contagious. Only a low amount of virus particles are necessary for infection, leading to either gastrointestinal disease (in case of norovirus) or liver infection (in the case of hepatitis A virus). Thus, the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit was designed for a high level of sensitivity with consistent specificity. Because both viruses are known for their food and water-related transmission routes, this kit was additionally designed and validated for the specific use and demands in food and water diagnostics. To ensure maximum reliability of the kit and to prevent misinterpretation of negative results, due to potential amplification inhibition by diverse sample matrices (e.g. soft fruits, vegetables, shellfish, fish, minced meat, and water), the Process Control (vial 3, white cap, contains bacteriophage MS2) must be added to the examined sample at the beginning of sample processing. The viral RNA is extracted using the foodproof Sample Preparation Kit IV (KIT230185) and subsequently analyzed with the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit.

The same already transcribed RNA (now cDNA) of this preparation control is used as “Internal Amplification Control” in the Negative Control (vial 5, orange cap) and in the Control Template (vial 4, purple cap). A hydrolysis probe was designed to bind specifically to the Process Control, allowing detection in the Cy5 channel, whereas the RNA of hepatitis A virus is detected in the FAM channel, norovirus GI in the HEX channel, and norovirus GII in the ROX channel.

***Note***: More detailed information is listed in the Validation Data Report of the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit. Please contact our Technical Support (www.hygiena.com/support).

### 1.4 Applicability Statement

The foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit is intended for food and water testing purposes. It is used to identify purified norovirus (GI, GII) RNA and hepatitis A virus RNA prepared and purified using the foodproof Sample Preparation Kit IV (KIT230185).

### 1.5 Background Information

Norovirus genus is a member of the *Caliciviridae* family, contains an RNA genome and is divided into five genogroups (GI-V) [see reference 8]. From these genogroups, GI and GII are responsible for most clinical infections in humans, whereby GII.4 has been found to be the most common. Noroviruses are the leading cause of outbreaks and sporadic cases of non-bacterial gastroenteritis worldwide. [6, 7] Beside person-to-person transmission, food and drinking water are considered important sources of this viral infection causing 14% of norovirus outbreaks worldwide [7].

Hepatitis A virus is a small, non-enveloped virus and a member of the *Picornaviridae* family. The genome is a single, positive-stranded RNA. Currently, the virus is divided into seven genotypes, whereby only genotypes I – III are known to infect humans [2]. It can cause a liver infection, but it also often causes only mild or asymptomatic disease. Several food related outbreaks of hepatitis A virus have been reported [1, 3]. Therefore, the same analyzing methods can be used for both viruses [4]. Since cell culture methods are time-consuming and not very sensitive (or not available, like for noroviruses), the reverse transcriptase-PCR has become the method of choice and is used as the gold-standard [4]. Detection of hepatitis A virus or norovirus particles requires 3 steps according to the ISO 15216: virus concentration from the sample using a Process Control (step 1, using ISO protocols for liberation of viruses from the test sample), RNA extraction (step 2, use our foodproof Sample Preparation Kit IV kit) and real-time RT-PCR (step 3, possible with this kit).

## 2. How to Use this Product

### 2.1 Before You Begin

#### *2.1.1 Precautions*

Detection of hepatitis A virus and norovirus RNA using the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit requires RNA transcription to DNA and DNA amplification by PCR. The kit provides all reagents required for the reverse transcription and for real-time PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease-, carry-over-, or cross-contamination:

- Prepare appropriate aliquots of the kit solutions and keep them separate from other reagents in the laboratory.
- Use nuclease-free labware (e.g., pipettes, pipette tips, reaction vials).
- Wear powder-free gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use sterile aerosol-preventive pipette tips.
- To avoid carry-over contamination, transfer the required solutions for one experiment into a fresh tube, rather than directly pipetting from stock solutions.
- Physically separate the workplaces for RNA preparation, PCR setup, and PCR to minimize the risk of carry-over contamination. Use a PCR hood for all pipetting steps.

Note: Protect the Master Mix (vial 1, yellow cap) from light and avoid multiple freezing and thawing cycles.

### *2.1.2 Additional Equipment and Reagents Required*

- Real-time PCR instrument suitable for detection of FAM-, VIC/HEX-, ROX-, and Cy5-labeled probes
- Real-time PCR compatible tubes, strips or plates with optical cap or foil applicable for the PCR cycler in use
- foodproof Sample Preparation Kit IV (Product No. KIT230185)
- nuclease-free, aerosol-resistant pipette tips
- pipettes
- sterile reaction tubes for preparing PCR mixes and dilutions
- powder-free gloves

### *2.1.3 Sample Material*

Use any sample material (RNA) suitable for real-time RT-PCR in terms of purity, concentration, and absence of inhibitors. For food or water samples use the corresponding ISO protocol for virus concentration (add Process Control to the sample). For preparation of genomic RNA from raw material use our foodproof Sample Preparation Kit IV.

### *2.1.4 Assay Time Real-time RT-PCR*

| Procedure | Time |
|---|---|
| PCR Setup | 15 min |
| PCR run | 140 min (*time may vary based on real-time PCR instrument)* |
| **Total assay time** | **155 min** |

### *2.1.5 Positive Control*

Always run a positive control with the samples. To prepare a positive control, replace the template RNA with the provided positive control DNA [foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit - Control Template (vial 4, purple cap)] or with a positive sample preparation control.

### *2.1.6 Negative Control*

Always run a negative control with the samples. To prepare a negative control, replace the template RNA with the provided negative control [foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit – Negative Control (vial 5, orange cap)]. It contains a stabilized solution of DNA of the Process Control.

### *2.1.7 PCR-Grade Water*

Optional: Include a negative PCR control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as a second negative PCR control.

Optional: Replace the template RNA with the provided PCR-grade $H_2O$ (vial 6, colorless cap) to create a RNA-free sample.

### *2.1.8 Process Control*

You cannot use the Process Control provided in the kit directly as template for real-time RT-PCR. The vial contains a stabilized solution of bacteriophage MS2. You must first perform a RNA extraction.

Always run the Process Control (vial 3, white cap) together with the samples. To prepare a Process Control, pipette 10 µL of the control virus to the sample in the first step of the RNA preparation procedure (see foodproof Sample Preparation Kit IV). For most sample matrices, a virus pre-concentration step is necessary (e.g., for soft fruits and vegetables, bottled water and bivalve molluscan shellfish). The Process Control has to be added at the start of sample processing.

For RNA preparation of the Process Control without a sample pipette 10 µL of the Process Control into 130 µL $H_2O$ PCR -grade in the first step of the RNA preparation procedure (see foodproof Sample Preparation Kit IV, protocol for 140 µL samples) and perform the procedure as with a normal sample.

***Note regarding calculation of the recovery rate*****:** We recommend to use the delta-Cq-method for calculation of the recovery rate to limit the sample numbers. Therefore add 10 µl of Process Control RNA to the real-time RT-PCR and calculate the recovery rate with the results of two samples: First use the Cq value of the Process Control in the food or water sample. Second use the Cq value of the Process Control treated without step 1 of the ISO 15216.

Please do not hesitate to contact our customer service for any further questions.

### 2.2 Program Setup

Program the PCR instrument before preparing the reaction mixes. The amplification is carried out according to the following temperature-time-program (for details on how to program the experimental protocol, see the operation manual of your real-time PCR cycler):

| | | |
|---|---|---|
| Reverse transcription | 1 cycle | Step 1: 45 °C for 30 minutes |
| Pre-Incubation | 1 cycle | Step 1: 95 °C for 5 minutes |
| Amplification | 50 cycles | Step 1: 95 °C for 15 seconds<br>Step 2*: 60 °C for 60 seconds<br>Step 3: 72 °C for 10 seconds |

*Fluorescence detection in step 2

***Note***: For some real-time PCR instruments (e.g. ABI 7500) the type of probe quencher as well as the use of a passive reference dye must be entered. The foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit contains probes with a non-fluorescent quencher and no passive reference dye. For users of the Agilent Mx3005p instrument: Click ‘Instrument Filter Set Gain Settings’ to open the Filter Set Gain Settings dialog box in which the gain settings may be viewed and modified. For FAM and HEX the Filter Set Gain Setting must be modified to ‘x4’.

### 2.3 Preparation of the Real-time RT-PCR Mix

Proceed as described below to prepare a 25 µL standard reaction.
Always wear gloves when handling the PCR vessels.

1. Completely thaw the Master Mix (vial 1, yellow cap) at room temperature (15 to 25 °C). For maximal recovery of contents, briefly spin vials in a microcentrifuge before opening. Mix carefully but thoroughly by pipetting up and down. Do not use a vortex.
2. We recommend the use of a cooling block (at 4 °C) to avoid degradation effects of the Enzyme Solution (vial 2, red cap).

3. In a reaction tube (0.5 – 2.0 mL, depending on the number of reactions), prepare the PCR mix by adding the following components in the order mentioned below, then mix gently but thoroughly by pipetting up and down. Do not use a vortex.
4. The volumes indicated below are based on a single 25 µL standard reaction. Prepare the RT-PCR mix by multiplying the amount in the "Volume" column by the number of reactions (sample and control reactions) to be cycled plus one or two additional reactions to cover pipetting losses. You need at least one additional sample for RNA of the Process Control if you use the delta-Cq-method for calculation of the recovery rate.

| Components of the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit | Volume |
|---|---|
| Master Mix, (vial 1, yellow cap) | 14 µL |
| Enzyme Solution, (vial 2, red cap) | 1 µL |
| **Total volume** | **15 µL** |

5. Reaction Mix Preparation
   - Pipette 15 µL PCR mix into each PCR vessel.
   - For the samples of interest, add up to 10 µL sample RNA (if less than 10 µL add $H_2O$ (vial 6, colorless cap)
   - to bring to 10 µLl).
     ***Note:*** Thaw RNA only on ice or at 4 °C in a cooling block
   - For the negative control, add 10 µL Negative Control (vial 5, orange cap).
   - For the positive control, add 10 µL Control Template (vial 4, purple cap).

6. Seal the PCR vessels accurately with optical caps or sealing foil.
7. Briefly spin the PCR vessels in a suitable centrifuge.
8. Load the PCR instrument and cycle the samples as described above.

**2.4 Data Interpretation**

The amplification of the hepatitis A Virus RNA is analyzed in the fluorescence channel suitable for FAM-labeled probes. Norovirus RNA can be detected in two channels: Norovirus GI is analyzed in the HEX channel and norovirus GII in the ROX channel. The specific amplification of the Process Control is analyzed in the Cy5 fluorescence channel.

Compare the results from the FAM channel (hepatitis A virus), HEX channel (norovirus GI), ROX channel (norovirus GII) and Cy5 channel (Process Control) for each sample, and interpret the results as described in the table below.

| Hepatitis A Virus<br><br>Channel FAM | Norovirus GI<br><br>Channel HEX | Norovirus GII<br><br>Channel ROX | Process Control<br><br>Channel Cy5 | Result Interpretation |
|---|---|---|---|---|
| Positive | Positive | Positive | Positive* / Negative | Positive for hepatitis A virus, norovirus GI and GII<br>*Note: Expected result of the Control Template |
| Positive | Positive | Positive | Positive | Expected result of the Control Template |
| Positive | Positive | Negative | Positive / Negative | Positive for hepatitis A virus and norovirus GI; negative for norovirus GII |
| Positive | Negative | Positive | Positive / Negative | Positive for hepatitis A virus and norovirus GII; negative for norovirus GI |
| Negative | Positive | Positive | Positive / Negative | Positive for norovirus GI and GII; negative for hepatitis A virus |
| Positive | Negative | Negative | Positive / Negative | Positive for hepatitis A virus; negative for norovirus GI and GII |
| Negative | Positive | Negative | Positive / Negative | Positive for norovirus GI; negative for hepatitis A virus and norovirus GII |
| Negative | Negative | Positive | Positive / Negative | Positive for norovirus GII; negative for hepatitis A virus and norovirus GI |
| Negative | Negative | Negative | Positive | Negative for hepatitis A virus, norovirus GI and GII<br>Note: Expected result of the Negative Control. |
| Negative | Negative | Negative | Negative | Invalid<br>*Note*: Expected result of the no-template control (H2O PCR-grade, vial 6 colorless cap). |

***Note**:* A prerequisite for the unambiguous detection of norovirus GI and GII, hepatitis A Virus- as well as the Process Control RNA in this multi-color experiment is a suitable calibration of the PCR Instrument for channels FAM, HEX, ROX, and Cy5. Please refer to the operation manual of your real-time PCR cycler for further information. A Color Compensation (Color Compensation Set 3; Product No. KIT230005) is necessary and is available for users of the LC 480 Systems I and II. Please contact Hygiena Diagnostics for further information.

## 3. Troubleshooting

| Observation | Possible Reason | Recommendation |
|---|---|---|
| No signal increase is observed, even with positive controls. | Incorrect detection channel has been chosen. | • Set Channel settings to FAM, HEX, ROX, and Cy5 |
| | Pipetting errors or omitted reagents. | • Check for correct pipetting scheme and reaction setup. Repeat the PCR run.<br>• Always run a positive control along with your samples. |
| | No data acquisition programmed. | • Check the cycle programs. |
| | Inappropriate storage of kit components. | • Store the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) at -15 to -25 °C, protected from light.<br>• Avoid repeated freezing and thawing. |
| | Inappropriate mixing of Master Mix and Enzyme Solution | • Mix carefully but thoroughly by pipetting up and down after the Master Mix is completely thawed.<br>• Do not vortex at any time! |
| No signal increase in any fluorescence channel for the sample, even in Cy5 for the Process Control is observed. | Inhibitory effects of the sample material (e.g., caused by insufficient purification). | • Use the recommended RNA sample preparation kit to purify template RNA.<br>• Dilute samples 1 to 10 (e.g. 5 µL sample to 45 µL $H_2O$). |
| Fluorescence intensity is too low. | Inappropriate storage of kit components. | • Store the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) at -15 to -25 °C, protected from light.<br>• Avoid repeated freezing and thawing. |
| | Inappropriate mixing of Master Mix and Enzyme Solution | • Mix carefully but thoroughly by pipetting up and down after the Master Mix is completely thawed.<br>• Do not vortex at any time! |
| | foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) is not homogeneously mixed. | • Mix the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) and the entire PCR mix thoroughly before pipetting. |
| | Low initial amount of target RNA. | • Increase the amount of sample RNA. Depending on the chosen RNA isolation method, inhibitory effects may occur. |
| Negative control samples are positive. | Carry-over contamination. | • Exchange all critical solutions.<br>• Repeat the complete experiment with fresh aliquots of all reagents.<br>• Always handle samples, kit components and consumables in accordance with Good Laboratory Guidelines<br>• Add positive controls after sample and negative control |

| Observation | Possible Reason | Recommendation |
|---|---|---|
| Fluorescence intensity varies. | Insufficient centrifugation of the PCR vessels. Prepared PCR mix is still in the upper part of the vessel. | • Always centrifuge reaction vessels prior to loading the samples into the real-time PCR cycler |
| | Outer surface of the vessel or the seal is dirty, e.g., by direct skin contact. | • Always wear powder-free gloves when handling the vessels, caps and seals. |
| Precipitation of the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) | Incomplete thawing of the Master Mix the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) | • Warm up the Master Mix carefully in your hands and snap gently to the tube or mix carefully but thoroughly by pipetting up and down until the precipitation is completely gone. Do not vortex! |
| | Precipitation of stabilizing reagents in the foodproof Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) | |

## 4. References

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## 5. Supplementary Information

### 5.1 Ordering Information

Hygiena Diagnostics offers a broad range of reagents and services. For a complete overview of our products and for more information, please visit our website at www.hygiena.com.

### 5.2 Trademarks

**food**proof®, **micro**proof®, **vet**proof®, ShortPrep®, StarPrep®, RoboPrep® and LyoKit® are registered trademarks of Hygiena Diagnostics GmbH. Other brand or product names are trademarks of their respective holders.

### 5.4 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics, please contact our Technical Support staff (www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

### 5.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: R 302 50

## 6. Change Index

*Version 1, April 2018:*
Initial version

*Revision A, September 2023:*
Rebranding and new layout.
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