foodproof® Norovirus Detection Kit (GI, GII)

Revision A, December 2023

RT-PCR kit for the qualitative detection of norovirus and MS2-RNA using real-time instruments.

Product No. KIT230055

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

Table of Contents

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.

1.2 Storage and Stability

- **Avoid repeated freezing and thawing!**
- **Store the kit at –15 to –25 °C (in a freezer) until the expiration date printed on the label.**
- **If possible, work with a cooling box on the lab bench with the enzyme mix.**
- Avoid working with the enzyme mix for more than 5 min at room temperature, and do not thaw and freeze more than 5 times.

1.3 Kit Contents

Vial	Label	Contents / Function / Storage
1 yellow cap	foodproof® Norovirus Detection Kit Master Mix	• 2 x 500 µL • Ready-to-use primer and hydrolysis probe mix specific for norovirus genogroup I, II, and the Process Control / Internal Amplification Control • For amplification and detection of norovirus sequences of GI and GII • Store at –15 to –25 °C • **Protect from light!**
2 red cap	foodproof Norovirus Detection Kit Enzyme Solution	• 2 x 40 µL • Contains Reverse Transcriptase, Taq DNA Polymerase and a yellow dye for better visualization • Store at –15 to –25 °C. A high-quality freezer is very important • Reverse transcriptase is a very temperature-sensitive enzyme. After use, store the enzyme solution immediately at –20 °C
3 white cap	foodproof Norovirus Detection Kit Process Control	• 3 x 250 µL • Contains a stabilized solution of MS2 phage • For use as preparation / internal amplification control • Added to the samples at the beginning of the protocol • Store at –15 to –25 °C. A high-quality freezer is very important • Only thaw RNA on ice or in a 4 °C cooling block!
4 purple cap	foodproof Norovirus Detection Kit Control Template	• 1 x 140 µL • Contains a stabilized solution of DNA specific for norovirus genogroup I, II, and the Process Control • For use as run positive control with internal amplification control • Store at –15 to –25 °C.
5 orange cap	foodproof Norovirus Detection Kit Negative Control	• 1 x 140 µL • Contains a stabilized solution of DNA of the Process Control • For use as run negative control with internal amplification control • Store at -15 to -25 °C

Vial	Label	Contents / Function / Storage
6 colorless cap	foodproof Norovirus Detection Kit H_2O, PCR-grade	• 3 x 1 mL • Nuclease-free, PCR-grade H_2O. For use as dilution reagent • After first thawing, store at 2 to 8 °C

1.4 Product Description

The foodproof Norovirus Detection Kit is a one-step real-time reverse transcriptase PCR for the simultaneous, qualitative detection and differentiation of noroviruses of the genogroup I and II (GI and GII), as well as a process control / internal amplification control for a comprehensive and fast interpretation of the 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 Detection Kit is based on primer, probes, and methods that are mentioned in the ISO/TS 15216 and § 64 LFGB [5].

Since less than 100 virus particles are sufficient for an infection with the norovirus, the kit was designed for high-level sensitivity with consistent specificity in different matrices, like food, water and stool samples.

ISO/TS 15216 states that a Process Control must be used in procedures for measuring virus concentration in food and water samples. The foodproof Norovirus Detection Kit (GI, GII) contains an MS2 phage, a non-GMO single-strand RNA virus, as a Process Control, which additionally allows calculation of the virus recovery rate in compliance with ISO/TS 15216.

To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to inhibition of the amplification by diverse sample matrices, the Process Control (vial 3, white cap, contains MS2 phage) must be added to the examined sample at the beginning of sample processing. The viral RNA must be extracted by the foodproof Sample Preparation Kit IV (Product No. KIT230185) and can subsequently be analyzed by the foodproof Norovirus Detection Kit.

The same already-transcribed RNA (now DNA) of this preparation control is already added as the “Internal Amplification Control” to the Negative Control (vial 5, orange cap) and the Control Template (vial 4, purple cap). The hydrolysis probe was designed to bind specifically to the Process Control, allowing detection in the ROX channel, whereas the norovirus genogroup I RNA is detected in the HEX channel and norovirus genogroup II RNA in the FAM channel.

1.5 Application

The foodproof Norovirus Detection Kit is intended for food and water testing purposes and is also suitable for stool samples. It is used to identify purified norovirus RNA prepared and purified by the foodproof Sample Preparation Kit IV (Product No. KIT230185).

1.6 Product Characteristics

Specificity	The primers and hydrolysis probes (5'-nuclease probes) provided in the Master Mix, (vial 1, yellow cap) are sequence-specific for norovirus GI, GII and the Process Control. Specificity of the assay was proven with 15 norovirus and 11 non-target virus species, as well as 10 stool samples, negative for norovirus infection.
Sensitivity	The limit of detection was determined at 3 viral copies per reaction of norovirus GI and GII. Diagnostic sensitivity was proven by known norovirus positive samples, like stool, shellfish, minced meat and soft fruits with different degrees of contamination levels.
Reproducibility	Reproducibility of Ct values with different real-time PCR instruments, including LightCycler® 480 II and Roche LightCycler 96 (Roche Diagnostics), Mx3005p (Agilent), ABI 7500 FAST (Applied Biosystems®), PikoReal™ (Thermo Scientific), and iQ™ 5 Cycler (Bio-Rad). Variation of results was measured at 0.8 % for GI and 1.0 % for GII for these instruments with 30 copies per reaction.

Note*:* More detailed information is listed in the *Validation Data Report* for the foodproof Norovirus Detection Kit. Please contact our Technical Support at www.hygiena.com/support.

1.7 Background Information

Norovirus genus is a member of the *Caliciviridae* family, contains an RNA genome and is divided into five genogroups (GI – V) [see references 1, 2, 3]. Of these genogroups, GI and GII are responsible for most clinical cases in humans, with GII.4 the most common genotype. Noroviruses are the leading cause of outbreaks and sporadic cases of non-bacterial gastroenteritis worldwide [2, 4]. Beside person-to-person transmission, food is considered as an important source of this viral infection, causing 14 percent of norovirus outbreaks worldwide [1, 3, 6]. Due to the absence of a routine culturing method and other rapid, sensitive, highly specific methods, the reverse transcriptase-polymerase chain reaction has become the method of choice and is used as the gold standard [5].

2. Procedure

2.1 Before You Begin

2.1.1 Precautions and Warnings

Detection of norovirus RNA using the foodproof Norovirus Detection Kit requires RNA transcription to DNA and DNA amplification by PCR.

The kit provides all reagents required for reverse transcription and real-time PCR. However, to achieve reliable results, the entire procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease-, carryover- 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 carryover 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 carryover 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- and ROX-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)
- Pipettes
- Nuclease-free, aerosol-resistant pipette tips
- Sterile reaction tubes for preparing PCR mixes and dilutions
- Powder-free gloves

2.1.3 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration and absence of inhibitors. For preparation of genomic RNA from raw material, refer to the corresponding product package inserts of a suitable sample preparation kit (see *Additional Equipment and Reagents Required* above).

2.1.4 Assay Time

Procedure	Time
PCR setup	15 min
PCR run	140 min (e.g., LightCycler 480 II)
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 control DNA [foodproof Norovirus 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 no template control [foodproof Norovirus Detection Kit – Negative Control (vial 5, orange cap)]. Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.

2.1.7 Process Control

Always run the Process Control (vial 3, white cap) together with the samples. To prepare a Process Control, pipet 10 µL of the control virus to the sample at the first step of the RNA preparation procedure [see foodproof Sample Preparation Kit IV, Product No. KIT230185]. For some sample matrices, a virus pre-concentration step is necessary (e.g., for soft fruits and food vegetables, bottled water and bivalve molluscan shellfish; DIN CEN ISO/TS 15216). For that, the Process Control must be added at the start of sample processing.

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 Step 2*: 60 °C for 60 seconds 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 the probe quencher as well as the use of a passive reference dye must be determined. The foodproof Norovirus Detection Kit contains probes with a non-fluorescent ("dark") 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. For FAM and HEX, the Filter Set Gain Setting must be modified to "x4".

2.3 Preparation of the 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 foodproof Norovirus Detection Kit solutions, i.e., the Master Mix (vial 1, yellow cap) and Enzyme Solution (vial 2, red cap), at room temperature (~25 °C). For maximal recovery of contents, briefly spin vials in a microcentrifuge before opening. Mix carefully but thoroughly by pipetting up and down.
2. 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.

   The volumes indicated below are based on a single 25 µL standard reaction. Prepare the 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.

Component, foodproof Norovirus Detection Kit	Volume
Master Mix (vial 1, yellow cap)	14 µL
Enzyme Solution (vial 2, red cap)	1 µL
Total volume	15 µL

3. Prepare the reaction mixtures:
   - Pipet 15 µL of PCR mix into each PCR vessel.
   - For the samples of interest, add up to 10 µL sample RNA [if <10 µL add H_2O (vial 6, colorless cap) to 10 µL]. **Note:** Thaw RNA only on ice or at 4 °C in a cooling block.
   - For the negative control, add 10 µL of foodproof Norovirus Detection Kit, Negative Control (vial 5, orange cap).
   - For the positive control, add 10 µL of foodproof Norovirus Detection Kit, Control Template (vial 4, purple cap).

4. Seal the PCR vessels accurately with optical caps or sealing foil.

5. Briefly spin the PCR vessels in a suitable centrifuge.

6. Cycle the samples as described above.

2.4 Data Interpretation

The amplification of the norovirus **genogroup I**-specific target is analyzed in the fluorescence channel suitable for HEX-labeled probe detection and norovirus **genogroup II** in the fluorescence channel suitable for FAM-labeled probe detection. The specific amplification of the Process Control is analyzed in the fluorescence channel suitable for ROX.

Compare the results from FAM channel (genogroup II), HEX channel (genogroup I), and ROX channel (Process Control) for each sample, and interpret the results as described in the table below.

Norovirus GI HEX Channel	Norovirus GII FAM Channel	Process Control ROX Channel	Result Interpretation
Positive	Positive	Positive / Negative	Positive for norovirus genogroup I & II
Negative	Positive	Positive / Negative	Positive for norovirus genogroup II
Positive	Negative	Positive / Negative	Positive for norovirus genogroup I
Negative	Negative	Positive	Negative for norovirus genogroup I & II
Negative	Negative	Negative	Invalid

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

Check the results for each control and compare the results with the expected results as described in the table below.

Norovirus GI HEX Channel	Norovirus GII FAM Channel	Process Control ROX Channel	Expected results
Positive	Positive	Positive	foodproof Norovirus Detection Kit Control Template
Negative	Negative	Positive	foodproof Norovirus Detection Kit Negative Control
Negative	Negative	Positive	Process Control after sample processing and RNA extraction
Negative	Negative	Negative	foodproof Norovirus Detection Kit H_2O, PCR-grade

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 and ROX
	Pipetting errors or omitted reagents.	• Check for correct pipetting scheme and reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.
	No data acquisition programmed.	Check the cycle programs.
No signal increase is observed, even with the Process control (ROX).	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	• Use the recommended RNA sample preparation kit to purify template RNA. • Dilute samples 1 to 10 (e.g., 1 µL sample to 9 µL H_2O).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof Norovirus Detection Kit, Master Mix (vial 1, yellow cap) at –15 to –25 °C, protected from light. • Avoid repeated freezing and thawing.
	foodproof Norovirus Detection Kit, Master Mix (vial 1, yellow cap) is not homogeneously mixed.	Mix the 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.	Carryover contamination.	• Exchange all critical solutions. • Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carryover contamination. • Add positive controls after sample and negative control reaction vessels have been sealed.
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.
	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 and seal.
Precipitation of the foodproof Norovirus Detection Kit, Master Mix (vial 1, yellow cap)	Incomplete thawing of the Master Mix (vial 1, yellow cap)	Warm up the Master Mix carefully in your hands and tap the tube gently until the precipitation is gone (do not vortex!).
	Precipitation of stabilizing reagents in the Master Mix (vial 1, yellow cap)	

4. References

1. Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N and Katayama K (2003) Broadly Reactive and Highly Sensitive Assay for Norwalk-like Viruses Based on Real-Time Quantitative Reverse Transcription-PCR. *J Clin Microbiol*. 41:1548 – 1557.
2. Hoehne M and Schreier E (2006) Detection of Norovirus Genogroup I and II by Multiplex Real-Time RT-PCR Using a 3'-Minor Groove Binder-DNA Probe**.** *BMC Infect Dis*. 6:69.
3. Dreier J, Störmer M, Mäde D, Burkhardt S and Kleesiek K (2006) Enhanced Reverse Transcription-PCR Assay for Detection of Norovirus Genogroup I. *J Clin Microbiol*., 44:2714 – 2720.
4. Scherer K, Johne R, Schrader C, Ellerbroek L, Schulenburg J and Klein G (2010) Comparison of Two Extraction Methods for Viruses in Food and Application in a Norovirus Gastroenteritis Outbreak. *J Virol Methods*. 169:22 – 27.
5. Microbiology of Food and Animal Feed - Horizontal Method for Determination of Hepatitis A Virus and Norovirus in Food Using Real-Time RT-PCR - Part 2: Method for Qualitative Detection (ISO/TS 15216-2:2013); German version CEN ISO/TS 15216-2:2013.
6. Verhoef V, Hewitt J, Barclay L, Ahmed SM, Lake R, Hall AJ, Lopman B, Kroneman A, Vennema H, Vinjé J and Koopmans M (2015) Norovirus Genotype Profiles Associated with Foodborne Transmission, 1999–2012. *Emerg Infect Dis.* 21:592 – 599.

5. Supplementary Information

5.1 Ordering Information

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

5.2 License Notice

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5.4 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, contact our Technical Support staff (www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. 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 38.1

6. Change Index

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