For food testing purposes

FOR IN VITRO USE ONLY

foodproof® Norovirus Detection Kit (GI, GII, GIV) – 5´Nuclease –

Version 2, March 2017

PCR kit for the qualitative detection of norovirus RNA using real-time instruments

Order No. R 302 38
Kit for 64 reactions for a maximum of 62 samples

Store the kit at -15 to -25 °C
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1. Product Overview

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.

Storage and Stability

- Store the kit at -15 °C 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 Kit

<table>
<thead>
<tr>
<th>Vial</th>
<th>Label</th>
<th>Contents / Function / Storage</th>
</tr>
</thead>
</table>
| 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, GII, and GIV.  
  • Store at -15 to -25 °C.  
  • Avoid repeated freezing and thawing!  
  • Protect from light! |
| 2 red cap | foodproof® Norovirus Detection Kit Enzyme Solution | • 2 x 40 µl  
  • Contains Reverse Transcriptase and a yellow dye for better visualization.  
  • Store at -15 to -25 °C.  
  • Avoid repeated freezing and thawing!  
  • Protect from light! |
| 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  
  • Store at -15 to -25 °C.  
  • Only thaw RNA on ice or at 4 °C cooling block!  
  • Avoid repeated freezing and thawing! |
| 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 Process Control.  
  • For use as run positive control with internal amplification control.  
  • Store at -15 to -25 °C.  
  • Avoid repeated freezing and thawing! |
| 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.  
  • Avoid repeated freezing and thawing! |
| 6 colorless cap | foodproof® Norovirus Detection Kit H₂O PCR-grade | • 3 x 1 ml  
  • Nuclease-free, PCR-grade H₂O.  
  • For use as dilution reagent.  
  • After first thawing, store at +2 °C to +8 °C. |

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, 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 which 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 on sensitivity with consistent specificity in different matrices, like food and stool samples.

To ensure maximum reliability of the kit and to prevent misinterpretation of negative results, due to inhibition of the amplification by divers sample matrices, the Process Control (vial 3, white cap, contains MS2 phage) has to be added to the examined sample at the beginning of sample processing. The viral RNA has to be extracted by the foodproof® Virus Sample Preparation Kit (RDK 400 14) and can subsequently analysed by the foodproof® Norovirus Detection Kit.

The same already transcribed RNA (now DNA) of this preparation control is already added as “Internal Amplification Control” to the Negative Control (vial 5, orange cap) and the Control Template (vial 4, purple cap). A 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 channel HEX and norovirus genogroup II-RNA in channel FAM (for genogroup GI in FAM and HEX).

The foodproof® Norovirus Detection Kit minimizes contamination risk and contains all reagents (except for sample template RNA) needed for the detection of norovirus genogroup GI-, GII-, and GIV-RNA.
Application
The foodproof® Norovirus Detection Kit is intended for food testing purposes and also suitable for stool samples. It is used to identify purified norovirus RNA prepared and purified by the foodproof® Virus Sample Preparation Kit (RDK 400 14).

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, respectively. Specificity of the assay was proven by 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 10 viral copies per reaction of norovirus GI and GII, respectively. Diagnostic sensitivity was proven by known for norovirus positive samples like stool, shellfish, minced meat, and soft fruits with different degrees on contamination levels. |
| Reproducibility | Reproducibility of Ct-values with different real-time PCR-instruments, including Roche LightCycler® 480 II, Roche LightCycler® 96, Agilent Mx3005p, Applied Biosystems® 7500 FAST, Thermo Scientific PikoReal, and Bio-Rad iQ™ 5 Cycler. 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 of the foodproof® Norovirus Detection Kit. Please contact our Technical Support (bcd@bc-diagnostics.com).

Background Information
Norovirus genus is a member of the Caliciviridae family, containing an RNA genome and is divided into five genogroups (GI-V) [see references 1, 2, 3]. From these genogroups are GI and GII responsible for most clinical cases in humans, whereby GII.4 have been found to be the most common genotype at all. 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 by causing 14 percent of norovirus outbreaks worldwide [1, 3, 6]. Due to the absence of a routine method to culture these viruses, and no other rapid, sensitive and highly specific method existing, 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

Precautions and Warnings
Detection of \textit{norovirus} RNA using the \textit{foodproof} Norovirus 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.

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

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
• \textit{foodproof}® Virus Sample Preparation Kit \textsuperscript{1} (Order No. RDK 400 14)
• Nuclease-free, aerosol-resistant pipette tips
• Pipettes
• Sterile reaction tubes for preparing PCR mixes and dilutions
• powder-free gloves

\textsuperscript{1} Available from BIOTECON Diagnostics; see Ordering Information for details

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").

Assay Time

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Setup</td>
<td>15 min</td>
</tr>
<tr>
<td>PCR run</td>
<td>140 min (e.g. LC 480 II)</td>
</tr>
<tr>
<td>Total assay time</td>
<td>155 min</td>
</tr>
</tbody>
</table>

Positive Control
Always run a positive control with the samples. To prepare a positive control, replace the template RNA with the provided control DNA [\textit{foodproof}® Norovirus Detection Kit - Control Template (vial 4, purple cap)] or with a positive sample preparation control.

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 [\textit{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.

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 \textit{foodproof}® Virus Sample Preparation Kit, RDK 400 14]. 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 has to be added to the start of the 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 usage of a passive reference dye has to 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 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 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 solutions foodproof® Norovirus Detection Kit, 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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>foodproof® Norovirus Detection Kit, Master Mix , (vial 1, yellow cap)</td>
<td>14 µl</td>
</tr>
<tr>
<td>foodproof® Norovirus Detection Kit, Enzyme Solution, (vial 2, red cap)</td>
<td>1 µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>15 µl</td>
</tr>
</tbody>
</table>

3.  
• Pipet 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 H₂O (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 foodproof® Norovirus Detection Kit, Negative Control (vial 5, orange cap).  
• For the positive control, add 10 µl 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 probes detection and *norovirus genogroup II* in the fluorescence channel suitable for FAM labeled probes detection. The specific amplification of the Process Control is analyzed in the fluorescence channel suitable for ROX.

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

<table>
<thead>
<tr>
<th>Norovirus GI Channel HEX</th>
<th>Norovirus GII Channel FAM</th>
<th>Process Control Channel ROX</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive / Negative</td>
<td>Positive for <em>norovirus genogroup I &amp; II or GIV</em></td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive / Negative</td>
<td>Positive for <em>norovirus genogroup II</em></td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive / Negative</td>
<td>Positive for <em>norovirus genogroup I</em></td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative for <em>norovirus genogroup I &amp; II or GIV</em></td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Invalid</td>
</tr>
</tbody>
</table>

**Note:** A prerequisite for the unambiguous detection of *norovirus* target GI and GII as well as Process Control RNA in this multi-color experiment is a suitable calibration of the PCR Instrument for channels FAM, VIC/HEX, and ROX. Please refer to the operation manual of your real-time PCR cycler for further information. A Color Compensation (Color Compensation Set 3; Order No. A 500 10) is necessary and will be supplied by BIOTECON Diagnostics for users of the LC 480 Systems I and II. Please contact BIOTECON Diagnostics for further information.
3. Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Reason</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signal increase is observed, even with positive controls.</td>
<td>Incorrect detection channel has been chosen.</td>
<td>Set Channel settings to FAM, HEX, and ROX.</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors or omitted reagents.</td>
<td>• Check for correct pipetting scheme and reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.</td>
</tr>
<tr>
<td>No data acquisition programmed.</td>
<td>Inhibitory effects of the sample material (e.g., caused by insufficient purification).</td>
<td>• Use the recommended RNA sample preparation kit to purify template RNA. • Dilute samples 1 to 10 (e.g. 1 µl sample to 9 µl H₂O).</td>
</tr>
<tr>
<td>No signal increase any channel even in ROX for the Process control is observed.</td>
<td>Inappropriate storage of kit components.</td>
<td>• Store the foodproof® Norovirus Detection Kit, Master Mix (vial 1, yellow cap) at -15 °C to -25 °C, protected from light. • Avoid repeated freezing and thawing.</td>
</tr>
<tr>
<td></td>
<td>foodproof® Norovirus Detection Kit, Master Mix (vial 1, yellow cap) is not homogeneously mixed.</td>
<td>Mix the foodproof® Norovirus Detection Kit, Master Mix (vial 1, yellow cap) and the entire PCR-mix thoroughly before pipetting.</td>
</tr>
<tr>
<td></td>
<td>Low initial amount of target RNA.</td>
<td>Increase the amount of sample RNA. Depending on the chosen RNA isolation method, inhibitory effects may occur.</td>
</tr>
<tr>
<td>Negative control samples are positive.</td>
<td>Carry-over contamination.</td>
<td>• Exchange all critical solutions. • Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, kit components and consumables in accordance with the prepared standards. • Add positive controls after sample and negative control. • Reaction vessels have been sealed commonly accepted practices to prevent carry-over contamination.</td>
</tr>
<tr>
<td>Fluorescence intensity varies.</td>
<td>Insufficient centrifugation of the PCR vessels. Prepared PCR mix is still in the upper part of the vessel.</td>
<td>Always centrifugate reaction vessels.</td>
</tr>
<tr>
<td></td>
<td>Outer surface of the vessel or the seal is dirty, e.g. by direct skin contact.</td>
<td>Always wear powder-free gloves when handling the vessels and seal.</td>
</tr>
<tr>
<td>Precipitation of the foodproof® Norovirus Detection Kit, Master Mix (vial 1, yellow cap)</td>
<td>Incomplete thawing of the Master Mix in the foodproof® Norovirus Detection Kit, Master Mix (vial 1, yellow cap)</td>
<td>Warm up the Master Mix carefully in your hands and snap gently to the tube until the precipitation gone (do not vortex)!</td>
</tr>
<tr>
<td></td>
<td>Precipitation of stabilizing reagents in the foodproof® Norovirus Detection Kit, Master Mix (vial 1, yellow cap)</td>
<td></td>
</tr>
</tbody>
</table>

4. References

5. Supplementary Information

5.1 Ordering Information
BIOTECON Diagnostics offers a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.bc-diagnostics.com.

5.2 License Notice
The purchase price of this product includes limited, nontransferable rights under U.S. Patent No. 7,687,247 owned by Life Technologies Corporation to use only this amount of the product to practice the claims in said patent solely for activities of the purchaser for bioburden testing, environmental testing, food testing, or testing for genetically modified organisms (GMO) in accordance with the instructions for use accompanying this product. No other rights are conveyed, including no right to use this product for in vitro diagnostic, therapeutic, or prophylactic purposes. Further information on purchasing licenses under the above patent may be obtained by contacting the Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008. Email: outlicensing@lifetech.com.

5.3 Trademarks
foodproof® is a trademark of BIOTECON Diagnostics GmbH.
Other brand or product names are trademarks of their respective holders.

5.4 Contact and Support
If you have questions about this or any other product of BIOTECON Diagnostics, please contact our technical support staff via email or telephone. Our scientists commit themselves to providing rapid and effective support. 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.

6. Change Index
Version 1, April 2015
First version of the manual.
Version 2, March 2017
License Notice changed.