

REAGENTS PROVIDED

Each kit contains enough reagents to perform 96 tests. Each kit also contains a package insert.

For real time PCR

Ref.	Type of Reagent	Presentation	Cap Color
A1	Enzyme-Mix	1 vial, 90µl	blue
A2	Primer and Probe-Mix	1 vial, 1.4ml	yellow
A3	Positive Control	1 vial, 50µl	red
A4	Negative Control	1 vial, 200µl	green

Norovirus real time RT-PCR Kit (TaqMan)

Qualitative screening assay for the real time detection of Norovirus (genotype I and II) using real time PCR microtiter plate systems (e.g. Applied Biosystems, Roche LC480, Stratagene or Qiagen/Corbett Research).

Cat. No: 942100

For in vitro diagnostic use

96 Determinations

INTENDED USE

The AnDiaTec[®] Norovirus real time RT-PCR (TaqMan) Kit is a screening assay for the detection of Noroviruses in stool samples using microtiter plate systems (e.g. Applied Biosystems, Roche LC480, Stratagene, Qiagen/Corbett Research)

SUMMARY

Gastroenteritis may be caused by a variety of enteric viruses. Even in industrialized countries gastrointestinal infections can cause life threatening diseases ultimately leading to death. It was recently shown, that the genetic heterogenous group of Noroviruses (formally known as Norwalk-like viruses) are the major cause of the non bacterial gastroenteritis worldwide. The Center of Disease Control (CDC, Atlanta, GA, USA) estimates that 23 billion cases of gastroenteritis/year may be attributed to human caliciviridae (Mead et al. 1999). Thus, 66 % of all food- and water-borne infectious diseases are associated with Noroviruses. In contrast, only 30,2 % of infection diseases are of bacterial origin (5.2 million) or 2.6 % of parasite origin (Mead et al. 1999).

Human Noroviruses are small, non-enveloped viruses with a ss-RNA (single-stranded) genome. Noroviruses belong to the family of *Caliciviridae* and are divided into genotype I and II. These viruses are resistant against higher temperatures (60°C), acid (pH 3) and chlorit (10 mg/L). The viruses are transmitted via contaminated food and water but also from person-to-person and are highly contagious.

PRINCIPLE OF THE TEST

The AnDiaTec[®] Norovirus real time RT-PCR Kit (TaqMan) contains specific primers, probes and additional material for the detection of the Norovirus I and II in stool.

The first step of the Norovirus detection is a reverse transcription (RT), during which the Norovirus RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify Norovirus I or II specific gene fragments by means of PCR (polymerase chain reaction). Furthermore, proof of specificity is achieved by hybridization of the amplicons with probes specific for genotype I and II. Fluorescence is emitted and measured by the real time PCR machine's optical unit (e.g. ABI PRISM SDS, Roche LC480, Stratagene MxPro, Qiagen/Corbett Research Rotorgene 3000/6000 software). The RT and PCR are done in one step. The detection of amplified Norovirus fragment is performed in fluorimeter channel FAM.

To exclude a possible RT-PCR inhibition, the Primer and Probe-Mix contains an Internal Control. The amplification of this Internal Control does not affect the Norovirus amplification and is measured by a probe's VIC/ HEX fluorescence.

STORAGE AND HANDLING

All reagents (A1 to A4) should be stored at -20°C. All reagents can be used until the expiration date printed on the labels. Avoid multiple freezing and thawing cycles of reagents (< 2). If used sporadically, prepare aliquots of the reagents. Cool all reagents during the working steps. Avoid exposure of A2 to light.

ASSAY PROCEDURE

Required material provided:

- PCR reagents
- Package insert

Required material not provided:

1. ABI system or a comparable instrument (e.g. Roche LC480, Stratagene, Qiagen/Corbett Research)
2. TC II reaction plate, 96 wells (Applied Biosystems) or comparable optical microtiter plates or optical reaction tubes.
3. Optical adhesive covers (Applied Biosystems) or comparable covers
4. RNA extraction kit
5. Pipets (0.5µl - 1ml) with sterile filter tips
6. Sterile microtubes

WARNINGS AND PRECAUTIONS

- This assay needs to be carried out by skilled personnel!
- Clinical samples should be regarded as potentially infectious materials.
- This assay needs to be run according to GLP (Good Laboratory Practice).

AMPLIFICATION

The PCR technology is utmost sensitive. Thus, amplification of a single molecule generates millions of identical copies. These copies may evade through aerosols and sit on surfaces.

In order to avoid contamination of samples with RNA which was previously amplified, it is important to physically strictly divide sample and reagent preparation units from sample amplification units. Pipets, vials and other working materials should not circulate among working units!

- Do not use the kit after its expiration date
- Set up (if possible) two separate working areas:
 1. Isolation of the RNA
 2. Amplification/ detection of amplification products
- Always use sterile pipet tips with filter
- Wear separate coats and gloves in each area
- Routinely decontaminate your pipets and the laboratory benches with decontaminant
- Avoid aerosols

PROCEDURE

The complete procedure is separated into three steps:

1. Sample preparation and RNA extraction.
2. Amplification and combined detection of RNA fragments using reverse transcriptase and probes.
3. Interpretation of the results using the ABI: PRSIM SDS (respectively Roche: LC480, Stratagene: MxPRO-, Qiagen/Corbett Research: Rotorgene 3000/ 6000) software.

1. SAMPLE PREPARATION AND RNA EXTRACTION

- 1.1 Extract genomic RNA by use of a commercial RNA isolation kit from stool sample supernatants according to the manufacturer's instructions.
- 1.2 If the real time RT-PCR is not performed immediately, store extracted RNA at -20°C.

2. Norovirus real time RT-PCR Protocol

Please read carefully the manufacturer's instructions before starting the procedure! Each assay should include a Negative and Positive Control. Use filter tips for all pipetting.

- 2.1 The Enzyme-Mix volume per reaction/ sample should be multiplied by the number of samples (n), including the controls **A3** and **A4**. For reasons of unprecise pipetting, add an extra (virtual) sample. Proceed in the same manner with all additional reagents. **Cool all reagents during the working steps!**

<u>Reaction Volume</u>	<u>Master-Mix Volume</u>
0.8µl Enzyme-Mix (A1)	0.8µl x (n + 1)
14.2µl Primer and Probe-Mix (A2)	14.2µl x (n + 1)

Mix gently (do NOT vortex) the following reagents in a sterile tube: Enzyme-Mix (A1) and Primer and Probe-Mix (A2). This mixture is the Master-Mix. Spin down briefly in a table centrifuge.

- 2.2 Pipet **15µl** of the Master-Mix using micropipets with sterile filtertips in each of the wells of the microtiter plate.
Add **5µl** of sample RNA or Positive or Negative Control to each of these wells. Pipet the Negative Control first.
- 2.3 Cover the 96 well optical microtiter plate with optical adhesive cover.
- 2.4 Run the RT-PCR using the following temperature protocol:

45°C for 15 min

95°C for 2 min

45 cycles of:

95°C for 20 sec

53°C for 60 sec measurement at the end of this step

72°C for 20 sec

3. RT-PCR ANALYSIS AND INTERPRETATION OF THE RESULTS

Norovirus specific amplification is detected by FAM fluorescence. The Internal Control is measured by VIC/ HEX fluorescence. Use following settings to define a reporter and quencher with the real time PCR software:

<u>Detection</u>	<u>Reporter</u>	<u>Quencher</u>
Norovirus RNA	FAM	none
Internal Control	VIC/ HEX	none
Reference Dye		none

Following results can arise:

- 3.1 FAM fluorescence is detected:

The result is positive.

The sample contains Norovirus RNA.

The occurrence of VIC/HEX fluorescence is inessential as high concentrations of Norovirus RNA may reduce or even inhibit the amplification of the Internal Control.

- 3.2 No FAM, but VIC/ HEX fluorescence is detected:

The result is negative.

The sample does not contain Norovirus RNA.

The detected signal of the Internal control excludes the possibility of an inhibition of the RT-PCR.

- 3.3 Neither FAM nor VIC/ HEX fluorescence is detected:

A diagnostic statement cannot be made.

An inhibition of the RT-PCR occurred.

RELATED PRODUCTS

Norovirus real time RT-PCR Kit with Extraction Control ,
Cat. No. 942200:

Kit for the detection of Norovirus (genotype I and II) in real time PCR microtiter plate systems (Applied Biosystems, Roche LC 480, Stratagene or Qiagen/Corbett Research) including a separate Extraction Control.

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