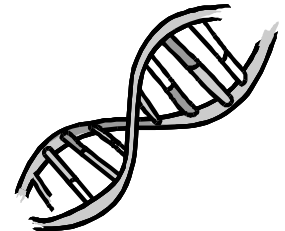



MutaPLATE[®]

Norovirus

real time RT-PCR Kit



Qualitative assay for specific *real time* detection of Norovirus (genotype I and II) using *real time* PCR microplate systems (e. g. Applied Biosystems, Stratagene, Corbett Research: RotorGene, Cepheid: SmartCycler).

REF KV1934196 



For in vitro diagnostic use



Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
www.immundiagnostik.com
info@immundiagnostik.com

Tel.: +49 (0)6251/ 701900
 Fax: +49 (0)6251/ 849430



1. INTENDED USE

The **MutaPLATE® Norovirus** *real time* RT-PCR kit is a qualitative assay for the specific detection of Norovirus (genotype I and II) in stool samples using microplate systems (e. g. Applied Biosystems, Stratagene, Corbett Research: RotorGene, Cepheid: SmartCycler, etc).

2. INTRODUCTION

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 heterogeneous group of Noroviruses (formally known as Norwalk-like viruses) are the major cause of 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 ssRNA (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.

3. PRINCIPLE OF THE TEST

The **MutaPLATE® Norovirus** *real time* RT-PCR kit contains specific primers, fluorescence-marked hybridisation probes and additional material for the detection of the Norovirus (genotype I and II). Stool samples are used as starting material for the extraction of RNA. Target sequence for the detection is the region in the ORF 1 / ORF 2 junction.

The amplification of possibly present *Norovirus* RNA and the proof of specificity by hybridization of the amplicon specific TaqMan probe is done in one step. The hybridisation prob is labelled with a fluorescence dye on one end and a quencher molecule on the other end. In case of a *Norovirus* specific amplicon, the emitted fluorescence signal is detected by the *real time* PCR microplate systems optical unit (ABI: PRISM SDS-, Stratagene: MxPRO-, Corbett Research: Rotorgene 3000/ 6000- Software).

Norovirus specific amplification is measured by **FAM** fluorescence (470 / 510 nm).

To exclude a possible RT-PCR inhibition, the amplification mix contains an internal control. The amplification of this internal control does not affect the *Norovirus* detection and is measured by a probe's **VIC / HEX / JOE** fluorescence (530/ 555 nm).

4. KIT CONTENT

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

Reference	Type of Reagent	Presentation	Cap Colour
A1	Enzyme Mix	1x 90 µl	blue
A2	Primer-/ Probe Mix	2 x 750 µl	yellow
A3	Positive Control	1 x 50 µl	red
A4	Negative Control	1 x 0.2 ml	green

5. TEST PERFORMANCE

Required materials - provided:

- PCR reagents
- Package insert

Required materials - not provided:

- ABI system or comparable instrument (e.g. Stratagene, Corbett Research, Cepheid)
- TC II reaction plate, 96 wells (Applied Biosystems) or comparable microtiter plates or reaction tubes to be used for optical detection within the two-parts holding frame (Applied Biosystems)
- Optical adhesive covers (Applied Biosystems) or comparable covers
- RNA extraction kit
- Pipets (0.5 µl – 200 µl) with sterile filter tips

6. 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.
- Do not freeze and thaw the reagents A1, A2, A3 and A4 several times. If used sporadically, prepare suited aliquots of the reagents and freeze again immediately.
- Cool all reagents during the working steps.
- Primer-/ Probe-Mix (A2) should be stored in the dark.

7. WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- 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).
- Do not use the kit after its expiration date.

AMPLIFICATION

The RT-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 previously was amplified, it is important to physically strictly divide sample and reagent preparation units from sample amplification units. Set up two separate working areas:

- 1) Isolation of the RNA
- 2) Amplification/ detection of amplification products

Pipets, vials and other working materials should not circulate among working units!

- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Routinely decontaminate your pipettes and laboratory benches with decontaminant.
- Avoid aerosols.

8. PROCEDURE

The complete procedure is separated in three steps:

- A) RNA extraction.
- B) Amplification and combined detection of RNA fragments using reverse transcriptase and hybridisation probes.
- C) Interpretation of the results using the ABI: PRISM SDS- (respectively the Stratagene: MxPRO-, Corbett Research: Rotorgene 3000/ 6000-) software.

A) RNA EXTRACTION

- 1) RNA extraction (by use of a commercial available RNA isolation kit):
Extract viral RNA from clinical stool samples by use of a commercial RNA isolation kit (suited for stool samples) according to the manufacturer`s instructions.
- 2) If *real time* RT-PCR is not performed immediately, store the extracted RNA samples at < -20°C.

B) **Real time Norovirus (MutaPLATE®) RT-PCR-PROTOCOL**

Please carefully read the manufacturer's instructions before starting the procedure!
Each assay should include a negative and positive control. Use filter tips for all pipetting:

- 1) The Enzyme Mix volume per reaction and sample (n) should be multiplied with the number of samples to be performed (including 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!**

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

- 2) Mix gently (**do NOT vortex!**) the following reagents in a sterile tube: Enzyme Mix (A1) and Primer-/ Probe Mix (A2). This mixture is the Master Mix. Spin down briefly in a table centrifuge.
- 3) Pipet **15 µl** of the amplification mix (Master Mix) per well of a 96 wells optical microtiter plate. The number of wells used is calculated from the number of samples plus one positive and one negative control.

Add **5 µl** of sample RNA or positive or negative control per well, respectively. Mix by up and down pipetting. Pipet the negative control first. To avoid contamination, it is advisable to cover the wells containing the negative control with an adhesive seal while pipetting the positive control and sample RNA. Remove this adhesive seal after preparing all wells.

Cover the 96 wells optical microtiter plate with optical adhesive cover.

- 4) Run the RT-PCR using following temperature protocol:

For RotorGene instrument choose following settings:

norovirus specific amplification: Source 470nm - Detector 510nm (FAM Channel)

Internal control amplification: Source 530nm - Detector 555nm (JOE Channel)

45°C for **30 min**

95°C for **2 min**

40 cycles:

95°C for **20 sec**

53°C for **60 sec**

72°C for **20 sec**

measurement at end of this step

C) RT-PCR ANALYSIS AND INTERPRETATION OF RESULTS

Use following settings to define a reporter and quencher with the ABI PRISM SDS software:

Detection	Reporter	Quencher
<i>Norovirus</i> RNA	FAM	none
Internal Control	VIC / HEX / JOE	none

Result interpretation is done till PCR cycle 38 (s. diagram below, end of log-phase positive control):

Norovirus specific amplification is detected by FAM fluorescence (at 510 nm).

The internal control is measured by VIC / HEX / JOE fluorescence (at 555 nm).

Following results can arise:

- 1) FAM fluorescence is detected.

The result is positive: The sample contains *Norovirus*.

The occurrence of VIC / HEX / JOE fluorescence is inessential as high concentrations of *Norovirus* RNA may reduce or even inhibit the amplification of the internal control.

- 2) No FAM, but VIC / HEX / JOE fluorescence is detected.

The result is negative: The sample does not contain *Norovirus*.

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

- 3) Neither FAM, nor VIC / HEX / JOE fluorescence is detected.

A diagnostic statement can not be made.

An inhibition of the RT-PCR reaction occurred.

