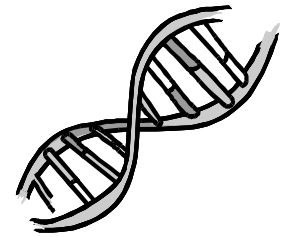


# MutaREAL<sup>®</sup> Norovirus


*real time RT-PCR Kit*





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Qualitative assay for the specific detection of **Norovirus** (genogroup I and II) in *real time* PCR capillary systems (e. g. LightCycler<sup>®</sup>, Roche<sup>\*</sup>).

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**\* MutaREAL<sup>®</sup> Norovirus is licensed from Roche Molecular Systems, Inc.**

For in vitro Diagnostic use only



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## 1. INTENDED USE

The **MutaREAL<sup>®</sup> Norovirus** *real time* RT-PCR kit is a qualitative assay for the specific detection of Norovirus (genogroup I and II) in stool samples using *real time* PCR capillary systems (e.g. LightCycler<sup>®</sup>, Roche) and **with license** from **Roche Molecular Systems, Inc.**.

## 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).

The reagents of the **MutaREAL<sup>®</sup> Norovirus** kit detect genera from high genetical diversity:

**GI:** *Norwalk, Southampton, Queens Arms, Desert Shield, Winchester, Sindlesham, Ciba*

**GII:** *Lordsdale, Melksham, Hawaii, Mexico, Leeds, Hillingdon, Snow Mountain, Toronto*

Human Noroviruses are small, non-enveloped viruses with **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). Due to their high **contagious** potential the viruses are transmitted **indirect** via contaminated food and water but also **direct** from person-to-person.

## 3. PRINCIPLE OF THE TEST

The **MutaREAL<sup>®</sup> Norovirus** *real time* RT-PCR kit contains specific primers, Fluorescence-marked probes and additional material for the detection of the Norovirus I and II. Stool samples are used as starting material for the extraction of RNA and the subsequent analysis with **MutaREAL<sup>®</sup> Norovirus**.

The first step of the Norovirus detection is a **reverse transcription** (RT), during which the viral ssRNA 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). **Target sequence** for the detection is the region in the **ORF1 / ORF2 - junction**.

Furthermore, proof of specificity is achieved by hybridization of the amplicons with fluorescence marked hybridisation probes specific for genotype I or II.

Fluorescence is emitted and measured by the LightCycler<sup>®</sup>'s optical unit during the PCR process. The RT and PCR are done in one step. The detection of **specific Norovirus amplicates** fragment is performed at **530 nm** (fluorimeter channel F1).

Furthermore, by the use of an included **internal control** in each reaction, which is coamplified and detected, a possible inhibition of the **reverse transcription** (RT) or the **PCR** is determined. The detection of amplified internal control is performed at **705 nm** (fluorimeter channel F3 for 1.5 respectively F6 for 2.0).

## 4. KIT CONTENT

Each kit contains enough reagents to perform **24** respectively **96** tests. Each kit also contains a package insert.

Ref.	Reagent	Presentation 24	Presentation 96	Cap color
A1	Enzyme Mix	1 vial, 30 µl	1 vial, 90 µl	blue
A2	Primer-/ Probe Mix	1 vial, 400 µl	2 vials, 750 µl	yellow
A3	Positive Control	1 vial, 20 µl	1 vial, 50 µl	red
A4	Negative Control	1 vial, 200 µl	1 vial, 200 µl	green

## 5. TEST PERFORMANCE

*Required materials - provided:*

- Reagents for *real time* RT-PCR
- Package insert

*Required materials - not provided:*

- *Real time* PCR capillary system (e. g. LightCycler® instrument, **Roche**)
- *Real time* PCR reaction tubes (e. g. LightCycler® capillaries, **Roche**)
- Table centrifuge (e. g. LightCycler® capillary centrifuge, **Roche**)
- Cryocontainer (e. g. LightCycler® cooling block, **Roche**)
- **Color Compensation Kit** for the used *real time* PCR system
- RNA extraction kit for stool samples (e. g. High Pure Viral RNA Kit, **Roche**)
- Pipets (0.5 µl – 200 µl)
- sterile filter tips for micro pipets
- sterile microtubes

## 6. STORAGE AND HANDLING

- All reagents (A1 to A4) should be **stored at <-20°C till immediate use** and then thawed **carefully** (at 8°C in refrigerator).
- **Avoid several freeze / thaw** cycles for the reagents A1, A2 and A3 (if necessary prepare suited aliquots and freeze again **immediately**).
- During preparation of PCR perform all working steps in a cryo-container (e.g. Light Cycler® Cooling block) or **cool all reagents** in suited manner.
- Primer-/ Probe-Mix (A2) should be **stored in the dark (light protection)**.
- All reagents can be used until the expiration date (printed on the labels).

## 7. WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- **MutaREAL® Norovirus** is **licensed from Roche** – **no further taxes** (e. g. for „reported-results“) are necessary.
- This assay needs to be carried out by especially in molecular biology 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 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/ DNA 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/ DNA
- 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 the laboratory benches with decontaminant
- Avoid aerosols

## 8. PROCEDURE

The complete procedure is separated in three steps:

- A) RNA extraction (stool).
- B) Reverse transcription of the RNA and following amplification and combined detection of cDNA templates using fluorescence-marked hybridisation probes in one step.
- C) Interpretation of the results using the software of the *real time* PCR capillary system.

### A) RNA-EXTRACTION

- 1) RNA Extraction of viral RNA is done by use of a commercial available RNA isolation kit (e.g. High Pure Viral RNA kit, Roche) from stool samples according to the instructions of the manufacturer. As starting material use an appropriate aliquot of supernatant gained from a stool sample diluted with 1 ml of pure water (do not use any buffer) after the set-down of solid particles.
- 2) If the *real time* PCR is not performed immediately, the extracted RNA **must** be stored at **<-20°C** for storage.

## B) *Real time* Norovirus RT-PCR-PROTOCOL

Please **read** careful the manufacturer's instructions **before** starting the procedure! The Master Mix volume for the respective number of samples and controls should be pipetted as follows:

- 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
<b>0.8 µl</b> Enzyme Mix (A1)	<b>0.8 µl</b> x (n+1)
<b>14.2 µl</b> Primer-/ Probe Mix (A2)	<b>14.2 µl</b> x (n+1)

- 2) Mix gently (Enzyme Mix, A1 and Primer-/ Probe Mix, A2) in a sterile microtube **by pipetting several times** (about **15 – 20 x**, **do NOT vortex !**): This mixture is the Master Mix - spin down briefly in a table centrifuge for collection of the solution at the bottom of the tube.
- 3) Pipet **15 µl** of Master Mix using micropipets with sterile filter tips in each of the *real time* PCR reaction tubes (e. g. LightCycler<sup>®</sup> capillaries).

Add **5 µl** of the RNA sample or positive and negative controls (A3 and A4) to each of the corresponding PCR reaction tubes (it is recommended to lock the capillaries of the samples immediately after filling and to pipet the negative control first but to close last [as contamination control]).

- 4) Spin down briefly to collect reagents at the bottom of PCR reaction tubes (for capillaries use LightCycler<sup>®</sup> capillary centrifuge).
- 5) Perform the following ***real time* RT-PCR** protocol:

**45°C** for **10 min** (reverse transcription)

**95°C** for **2 min**

**40 cycles:** (amplification)

**95°C** for **0 sec**

**50°C** for **30 sec**

**72°C** for **20 sec**

ramping time: **20°C/sec** – acquisition mode here: **SINGLE**

NONE

NONE

**40°C** for **30 sec**

### C) PCR ANALYSIS AND INTERPRETATION OF RESULTS

- 1) Perform the *real time* RT-PCR (e. g. in the LightCycler® capillary system, **Roche**).
- 2) Switch **on** the **color compensation** filter (required because of the simultaneous use of several fluorescence-marked probes) by activating the field *Choose CCC File*.
- 3) Result interpretation is done till **maximum PCR cycle 40** (see diagram below: end of the log-phase from the positive control): the result of the **specific Norovirus amplification** is shown **at 530 nm** (channel F1), the internal control is shown at 705 nm (channel F3 respectively F6).

#### Following results can arise:

- A signal is detected at 530 nm (F1).  
The result is positive: **The sample contains Norovirus RNA.**  
In this case, the detection of a signal at 705 nm is inessential, as high concentrations of *Norovirus* cDNA can lead to a reduced or absent fluorescence signal of the internal control in channel F3 (competition).
- No signal is detected at 530 nm (F1), but only at 705 nm (F3, signal of the internal control).  
**The sample does not contain any Norovirus RNA.**  
The detected signal of the internal control excludes the possibility of an inhibition.
- Neither at 530 nm (F1) nor at 705 (F3) nm is a signal detected.  
**A diagnostic statement can not be made.**  
**Inhibition** of the *real time* RT-PCR reaction.

