altona DIAGNOSTICS

Instructions for use

AltoStar®
Norovirus RT-PCR Kit 1.5

05/2023 EN

AltoStar®

AltoStar®

Norovirus RT-PCR Kit 1.5

For research use only!

(RUO)

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1. Application

The AltoStar® Norovirus RT-PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection and differentiation of norovirus genogroup I (GI) and II (GII) specific RNA.

For research use only (RUO)! Not for use in diagnostic procedures.

2. Kit content

The AltoStar® Norovirus RT-PCR Kit 1.5 contains the following components:

Table 1: Kit components

Lid color	Component	Number of tubes	Nominal volume [µl/tube]
Blue	Master A[1)]	8	60
Purple	Master B[1)]	8	180
Red	PC Norovirus GI[2)]	2	250
Orange	PC Norovirus GII[2)]	2	250
White	NTC[3)]	2	250

[1)] Contains biological material of animal origin

[2)] Positive Control

[3)] No Template Control (negative control)

The AltoStar® Norovirus RT-PCR Kit 1.5 contains enough reagents to perform 96 reactions.

3. Storage and handling

- The AltoStar® Norovirus RT-PCR Kit 1.5 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored at -25 °C to -15 °C upon arrival.
- Do not exceed the following thaw-freeze-sequence for each master reagent tube: *Thaw 1 → Freeze 1 → Thaw 2*
- Do not exceed the following thaw-freeze-sequence for each Positive Control (PC) and No Template Control (NTC) tube: *Thaw 1 → Freeze 1 → Thaw 2 → Freeze 2 → Thaw 3 → Freeze 3 → Thaw 4*
- After thawing all components are stable for 5 hours at up to +30 °C.

4. Product description

The AltoStar® Norovirus RT-PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection and differentiation of norovirus genogroup I (GI) and II (GII) specific RNA.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labeled with fluorescent reporter and quencher dyes.

In addition to the norovirus GI and norovirus GII RNA specific amplification and detection systems the AltoStar® Norovirus RT-PCR Kit 1.5 includes oligonucleotides for the amplification and detection of an internal control (IC, AltoStar® Internal Control 1.5). For details refer to the instructions for use of the AltoStar® Internal Control 1.5.

Probes specific for norovirus GI RNA are labeled with the fluorophore Cy5 whereas probes specific for norovirus GII RNA are labeled with the fluorophore FAM™. The probe specific for the IC is labeled with a fluorophore (JOE™) detectable in the e.g. VIC™ channel.

Using probes linked to distinguishable dyes enables the parallel detection of norovirus GI and norovirus GII specific RNA and the IC in the corresponding detection channels of the real-time PCR instrument.

4.1 Component description

Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, PCR mediated amplification and detection of norovirus GI specific RNA, norovirus GII specific RNA and the IC (AltoStar® Internal Control 1.5) in one reaction setup.

The PC contains norovirus GI and norovirus GII specific RNA. It is used to verify the functionality of the norovirus GI and norovirus GII specific amplification and detection systems.

The NTC contains neither norovirus GI, nor norovirus GII specific RNA but does contain the IC template. The NTC is used as negative control for the norovirus GI and the norovirus GII RNA specific real-time PCR and indicates possible contamination of Master A and Master B.

4.2 Real-time PCR instruments

The AltoStar® Norovirus RT-PCR Kit 1.5 can be used with the following real-time PCR instruments:

- ABI Prism® 7500 SDS (Applied Biosystems)
- CFX96™ Deep Well Dx System (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)
- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)

NOTE

Ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.

5. Material required but not provided

The following additional instruments and consumables are required for use of the AltoStar® Norovirus RT-PCR Kit 1.5 but not provided with this product:

- Appropriate real-time PCR instrument (see chapter 4.2 Real-time PCR instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 6.1 Sample preparation)
- Vortex mixer
- Centrifuge (e.g. desktop centrifuge) for centrifugation of kit reagents
- Centrifuge for centrifugation of PCR plates
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

Reagents required but not included in the AltoStar® Norovirus RT-PCR Kit 1.5:

- AltoStar® Internal Control 1.5 (Order No. IC15-06)

6. Procedure

6.1 Sample preparation

Extracted RNA is the starting material for the AltoStar® Norovirus RT-PCR Kit 1.5. The quality of the extracted RNA has a profound impact on the performance of the product.

The AltoStar® Norovirus RT-PCR Kit 1.5 is configured for use with the AltoStar® Internal Control 1.5 (IC), which allows to control the sample preparation procedure (nucleic acid extraction) and the subsequent RT-PCR.

Add the IC during the lysis step of the nucleic acid extraction procedure.

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the sample. The IC should always be added to the sample/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 50 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 30 µl of IC per sample must be added into the sample/lysis buffer mixture.

For additional information and technical support regarding pre-treatment and sample preparation, contact altona Diagnostics technical support (see chapter 9. Technical support).

6.2 Master mix setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

Set up the master mix according to the following pipetting scheme:

Table 2: Pipetting scheme (master mix setup)

Number of reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume master mix	**20 µl**	**240 µl**

NOTE

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the sample.

6.3 Reaction setup

1. Pipette 20 µl of the master mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
2. Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (PC or NTC).

Table 3: Pipetting scheme (reaction setup)

Reaction setup	
Master mix	20 µl
Sample or control	10 µl
Total volume	**30 µl**

NOTE

Do not add the IC to the PC and the NTC reactions, respectively, provided with this product.

3. Make sure that at least 1 PC and 1 NTC is used per run.

4. Thoroughly mix the samples and controls with the master mix by pipetting up and down.
5. Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
6. Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1,000 x g (~ 3,000 rpm).

After completion of the PCR mix setup the RT-PCR mix in a sealed PCR plate is stable at room temperature (max. +30 °C) for max. 30 minutes.

7. Programming the real-time PCR instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, refer to the instructions for use of the respective instrument.

For detailed programming instructions regarding the use of the AltoStar® Norovirus RT-PCR Kit 1.5 on specific real-time PCR instruments, contact altona Diagnostics technical support (see chapter 9. Technical support).

7.1 Settings

Define the following basic settings:

Table 4: Run settings

Settings	
Reaction volume	30 µl
Ramp rate	Default
Passive reference*	ROX™

* If applicable

7.2 Fluorescence detectors (dyes)

Define the following fluorescence detectors (dyes):

Table 5: Fluorescence detectors

Target	Detector name	Reporter	Quencher
Norovirus GI specific RNA	Norovirus GI	Cy5	(None)
Norovirus GII specific RNA	Norovirus GII	FAM™	(None)
IC	Internal Control	JOE™	(None)

7.3 Temperature profile and dye acquisition

Define the following temperature profile and dye acquisition:

Table 6: Temperature profile and dye acquisition

	Stage	Cycle repeats	Acquisition	Temperature [°C]	Time [min:s]
Reverse transcription	Hold	1	-	55	20:00
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			Yes	55	00:45
			-	72	00:15

8. Data analysis

For basic information regarding data analysis on specific real-time PCR instruments, refer to the instructions for use of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the AltoStar® Norovirus RT-PCR Kit 1.5 on different real-time PCR instruments, contact altona Diagnostics technical support (see chapter 9. Technical support).

8.1 Interpretation of results

8.1.1 Qualitative analysis

Table 7: Qualitative analysis

Detection channel			Result interpretation
Cy5 (norovirus GI)	FAM™ (norovirus GII)	JOE™ (IC)	
+	-	+/-*	Norovirus GI specific RNA detected.
-	+	+/-*	Norovirus GII specific RNA detected.
-	-	+	Neither norovirus GI nor norovirus GII specific RNA detected. The sample does not contain detectable amounts of norovirus GI or norovirus GII specific RNA.
-	-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the IC in the JOE™ detection channel is not required for positive results in the Cy5 and/or the FAM™ detection channel. A high norovirus GI and/or norovirus GII RNA load in the sample can lead to reduced or absent IC signals.

9. Technical support

For customer support, contact altona Diagnostics technical support:

e-mail: **support@altona-diagnostics.com**

phone: **+49-(0)40-5480676-0**

10. Trademarks and disclaimers

AltoStar® (altona Diagnostics); ABI Prism®, QuantStudio™ (Applied Biosystems); CFX96™ (Bio-Rad); Rotor-Gene® (QIAGEN); LightCycler® (Roche); FAM™, JOE™, ROX™, VIC™ (Thermo Fisher Scientific).

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11. Symbols

Symbol	Explanation
RUO	Research use only
LOT	Batch code
CONT	Content
CAP	Cap color
REF	Catalogue number
NUM	Number
COMP	Component
	Consult instructions for use
	Contains sufficient for "n" tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
MAT	Material number
	Version
	Note: Information is given to the user that is useful but not essential to the task at hand.

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