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RealStar®
Norovirus RT-PCR Kit 2.0

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altona Diagnostics GmbH
Moerkenstr. 12
22767 Hamburg
Germany

phone +49 40 548 06 76 - 0
fax +49 40 548 06 76 - 10
e-mail info@altona-diagnostics.com

www.altona-diagnostics.com

RealStar®

Norovirus RT-PCR Kit 2.0

For use with

m2000rt (Abbott Diagnostics)

Mx 3005P™ QPCR System (Stratagene)

VERSANT™ kPCR Molecular System AD (Siemens)

ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)

LightCycler® 480 Instrument II (Roche)

Rotor-Gene™ 3000/6000 (Corbett Research)

Rotor-Gene Q5/6 plex Platform (QIAGEN)

CE

IVD For *in vitro* diagnostic use

REF Product No.: 052013

Σ 96 rxns

Store at -25°C ... -15°C

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1. Intended Use

The RealStar® Norovirus RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of RNA specific for Noroviruses belonging to the genogroup I (G I) and genogroup II (G II). Furthermore, the test allows the differentiation between Norovirus genogroup I and Norovirus genogroup II specific RNA.

2. Kit Components

Lid Color	Blue	Purple	Green	Red	Orange	White
Component	Master A	Master B	Internal Control	Positive Control Norovirus G I	Positive Control Norovirus G II	PCR grade Water
Number of Vials	4	4	1	1	1	1
Volume [µl/Vial]	120	360	1000	125	125	500

3. Storage

- The RealStar® Norovirus RT-PCR Kit 2.0 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 -20°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage at +4°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (chapter 6. Product Description)
- Appropriate nucleic acid extraction system or kit
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

⚠ ***Please ensure that instruments have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.***

5. Background Information

Noroviruses (NV), formerly known as Norwalk-like viruses, are responsible for the majority of non-bacterial acute gastroenteritis in humans in industrialized countries. The symptoms of vomiting and diarrhea occur after a short incubation time of 8 to 72 hours. NV are highly infectious. Infections with NV can either be caused by contaminated food and/or drinking water or person-to-person virus transmission. NV can cause large outbreak situations in settings of close human contact such as hospitals, nursing homes, cruise ships, etc.

The genus Norovirus belongs to the family of Caliciviridae. NV are single stranded RNA viruses, discovered 1972 by electron-microscopy. They are characterized by their high degree of genomic variability. NV have been classified into five genogroups (G I to G V) based on sequence comparison of the RNA polymerase and capsid region of the genome. Genogroups I, II, and IV are associated with infections in humans. To date, the genogroups G I and G II are subdivided into at least 8 and 17 genotypes, respectively.

In recent years, a substantial increase of NV outbreaks has been reported in Western Europe. To prevent further spreading of the causative agent during an outbreak situation an immediate application of hygiene measures as well as rapid and sensitive diagnostics is needed. Since NV of the genogroups I and II can not be grown in cell culture and since enzyme immunoassays were found to be insufficient sensitive and/or insufficient specific, RT-PCR has become the method of choice for the diagnosis of NV infections.

6. Product Description

The RealStar® Norovirus RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection and differentiation of human pathogenic Noroviruses of the genogroups I and II. The assay includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

The test is based on real-time RT-PCR technology, utilizing 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 labelled with fluorescent reporter and quencher dyes.

Probes specific for Noroviruses of the genogroup I are labelled with a fluorophore showing the same characteristics as Cy5, whereas the probes specific for Noroviruses of the genogroup II are labelled with the fluorophore FAM. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore JOE. Using probes linked to distinguishable dyes enables the parallel detection of Norovirus of the genogroup I and II and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The test consists of three processes in a single tube assay:

- Reverse transcription of target RNA to generate cDNA
- PCR amplification of target cDNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® Norovirus RT-PCR Kit 2.0 was developed and validated to be used with the following real-time PCR instruments:

- m2000rt (Abbott Diagnostics)
- Mx 3005P™ QPCR System (Stratagene)
- Versant™ kPCR Molecular System AD (Siemens)
- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene™ 3000/6000 (Corbett Research)
- Rotor-Gene Q 5/6 plex Platform (QIAGEN)

The RealStar® Norovirus RT-PCR Kit 2.0 consists of:

- Two Master reagents (Master A and Master B)
- Template Internal Control (IC)
- Two Positive Controls: Positive Control (Norovirus G I)
  Positive Control (Norovirus G II)
- PCR grade water

Master A and Master B reagents contain all components (buffer, enzymes, primers, and probes) to allow reverse transcription, PCR mediated amplification and target detection (Norovirus Genogroup I, Norovirus Genogroup II, and Internal Control) in one reaction setup.

7. Warnings and Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) specimen preparation, (ii) reaction setup and (iii) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Instructions for Use

8.1 Sample Preparation

Extracted RNA is the starting material for the RealStar® Norovirus RT-PCR Kit 2.0. The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following nucleic acid extraction systems and kits are recommended:

- KingFisher® Flex (Thermo Scientific) with ExtraStar® Purification Kit (altona Diagnostics)
- VERSANT™ Molecular System SP (Siemens)
- HighPure® Viral Nucleic Acid Kit (Roche)
- QIAamp® Viral RNA Mini Kit (QIAGEN)

If using a spin column based sample preparation procedure including washing buffers containing ethanol, an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid, is highly recommended.

NOTE

⚠ ***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

⚠ ***Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

8.2 Master Mix Setup

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

The RealStar® Norovirus RT-PCR Kit 2.0 contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

- If the IC is used as a RT-PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1	24
Master A	5 µl	120 µl
Master B	15 µl	360 µl
Internal Control	0,5 µl	12 µl
Volume Master Mix	**20,5 µl**	**492 µl**

- If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, the IC has to be added during the nucleic acid extraction procedure.

- No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added depends always and only on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added to the specimen/lysis buffer mixture.

NOTE

⚠ ***Never add the Internal Control directly to the specimen!***

- If the IC was added during the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1	24
Master A	5 µl	120 µl
Master B	15 µl	360 µl
Volume Master Mix	**20 µl**	**480 µl**

8.3 Reaction Setup

- 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.
- Add 5 µl of the sample (eluate from the nucleic acid extraction) or 5 µl of the controls (Positive or Negative Control).
- Make sure that each of the Positive Controls and at least one Negative Control is used per run.
- Thoroughly mix the samples and controls with the Master Mix by up and down pipetting.
- Close the 96-well reaction plate with an appropriate optical adhesive film and the reaction tubes with appropriate lids.
- Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

Reaction Setup	
Master Mix	20 µl
Sample or Control	5 µl
Total Volume	**25 µl**

9. Programming the Real-Time PCR Instruments

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the manual of the respective instrument.
For detailed programming instructions regarding the use of the RealStar® Norovirus RT-PCR Kit 2.0 on specific real-time PCR instruments please contact our Technical Support.

9.1 Settings

- Define the following settings:

Settings	
Reaction Volume	25 µl
Ramp Rate	Default
Passive Reference	None

9.2 Fluorescent Detectors (Dyes)

- Define the fluorescent detectors (dyes):

Detection	Detector Name	Reporter	Quencher
Norovirus G I specific RNA	NV G I	Cy5	(None)
Norovirus G II specific RNA	NV G II	FAM	(None)
Internal Control	IC	JOE	(None)

9.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Reverse Transcription	Hold	1	-	50 °C	10:00 min
Denaturation	Hold	1	-	95 °C	10:00 min
Amplification	Cycling	45	-	95 °C	0:15 min
			√	58 °C	0:45 min

10. Data Analysis

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

For detailed instructions regarding data analysis of the RealStar® Norovirus RT-PCR Kit 2.0 on different real-time PCR instruments please contact our Technical Support.

10.1 Validity of Diagnostic Test Run

10.1.1 Valid Diagnostic Test Run

For a **valid** diagnostic test run, the following control conditions must be met:

Control ID	FAM Detection Channel	Cy5 Detection Channel	JOE Detection Channel
Positive Control Norovirus G I	NEGATIVE	POSITIVE	POSITIVE
Positive Control Norovirus G II	POSITIVE	NEGATIVE	POSITIVE
Negative Control	NEGATIVE	NEGATIVE	POSITIVE

10.1.2 Invalid Diagnostic Test Run

A diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

Control ID	FAM Detection Channel	Cy 5 Detection Channel	JOE Detection Channel	Result Interpretation
A	POSITIVE	NEGATIVE	POSITIVE*	Norovirus Genogroup II specific RNA detected.
B	NEGATIVE	POSITIVE	POSITIVE*	Norovirus Genogroup I specific RNA detected.
C	NEGATIVE	NEGATIVE	POSITIVE	The sample does not contain detectable amounts of Norovirus Genogroup I or II specific RNA.
D	NEGATIVE	NEGATIVE	NEGATIVE	RT-PCR Inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

*Detection of the Internal Control in the JOE detection channel is not required for positive results either in the FAM detection channel or in the Cy5 detection channel. High Norovirus load in the sample can lead to reduced or absent Internal Control signal.

11. Performance Evaluation

Since Norovirus does not grow in culture, there is no quantified standard material available. Therefore, performance evaluated of the RealStar® Norovirus RT-PCR Kit was done by using RNA extracted from a Norovirus Genogroup I isolate (G I.3) and RNA extracted from a Norovirus Genogroup II isolate (G II.4). The extracted RNA was quantified via quantitative real-time PCR using quantified *in vitro* transcripts as quantification standards to generate a standard curve.

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® Norovirus RT-PCR Kit is defined as the concentration (copies per µl of the eluate) of Norovirus specific RNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity was determined by analysis of dilution series of the extracted RNA of a Norovirus Genogroup I isolate and a Norovirus Genogroup II isolate.

Table 1: RT-PCR results used for the calculation of the analytical sensitivity of the Norovirus genogroup I and II specific system of the RealStar® Norovirus RT-PCR Kit

Norovirus Genogroup I				Norovirus Genogroup II			
Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]	Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
10	24	24	100	10	24	24	100
3.16	24	24	100	3.16	24	24	100
1	24	24	100	1	24	24	100
0.316	24	22	91,6	0.316	24	23	95,8
0.1	24	17	70,8	0.1	24	17	70,8
0.03	16	6	37,7	0.03	24	14	58,3
0.01	16	0	0	0.01	24	0	0
NTC	16	0	0	NTC	24	0	0

The analytical sensitivity of the RealStar® Norovirus RT-PCR Kit for the detection of RNA specific for Norovirus of the Genogroup I was determined to be 0.33 copies/µl eluate ($p \leq 0.05$) and for the detection of RNA specific for Norovirus of the Genogroup II to be 0.25 copies/µl eluate ($p \leq 0.05$), by Probit analysis.

11.2 Analytical Specificity

The analytical specificity of the RealStar® Norovirus RT-PCR Kit is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against public available sequences to ensure that all relevant Norovirus genotypes will be detected.

The analytical specificity of the RealStar® Norovirus RT-PCR Kit was evaluated by testing a panel of genomic RNA extracted from different Norovirus isolates along with genomic DNA/RNA extracted from other gastrointestinal pathogens and commensal flora found in the intestine and stool.

Table 2: Organisms tested to demonstrate the analytical specificity of the RealStar® Norovirus RT-PCR Kit

	RealStar® Norovirus RT-PCR Kit		
Organisms (n=different Isolates)	Cy5 Channel (NV G I Detection)	FAM Channel (NV G II Detection)	JOE Channel (Internal Control)
Norovirus G I.3 (n=2)	Positive	Negative	Positive
Norovirus G I.7 (n=1)	Positive	Negative	Positive
Norovirus G II.4 (n=2)	Negative	Positive	Positive
Norovirus G IV.1 (n=1)	Negative	Positive	Positive
Rotavirus (n=4)	Negative	Negative	Positive
Sapovirus (n=1)	Negative	Negative	Positive
Astrovirus (n=1)	Negative	Negative	Positive
Hepatitis A Virus (n=1)	Negative	Negative	Positive
Hepatitis E Virus (n=1)	Negative	Negative	Positive
Cryptococcus spec. (n=1)	Negative	Negative	Positive
Entamoeba spec. (n=1)	Negative	Negative	Positive
Entamoeba histolytica (n=1)	Negative	Negative	Positive
Giardia lamblia (n=1)	Negative	Negative	Positive
Clostridium difficile (n=2)	Negative	Negative	Positive
Escherichia coli (n=1)	Negative	Negative	Positive
Salmonella spec. (n=2)	Negative	Negative	Positive
Campylobacter spec. (n=2)	Negative	Negative	Positive

The RealStar® Norovirus RT-PCR Kit did not cross-react with any of the specified organisms.

11.3. Diagnostic Evaluation

Diagnostic specificity and sensitivity of the RealStar® Norovirus RT-PCR Kit was evaluated by analysing 100 Norovirus positive and 40 Norovirus negative specimen from patients with diarrhea. All specimens were pretested using either molecular or immunodiagnostic tests.

Table 3: Evaluation results of the diagnostic sensitivity and specificity of the RealStar® Norovirus RT-PCR Kit

		RealStar®Norovirus RT-PCR Kit	
		POSITIVE	NEGATIVE
pretested	POSITIVE	100	0
	NEGATIVE	0	40

11.4 Precision

Precision data for the RealStar® Norovirus RT-PCR Kit were determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots).
The variability data are expressed in terms of standard variation, variance and coefficient of variation based on the C_t-values of low positive controls (3-fold LoD) for Norovirus Genogroup I and Genogroup II and the Internal Control. Eight replicates per sample and per experiment were analysed.

Table 4: Precision data of the Norovirus RT-PCR Kit

Precision		Standard Deviation	Variance	Coefficient of Variation (%)
Intra-Assay Variability	Norovirus G I	0.80	0.64	2.36
	Norovirus G II	0.45	0.20	1.50
	Internal Control	0.07	0.01	0.34
Inter-Assay Variability	Norovirus G I	0.79	0.63	2.34
	Norovirus G II	042	0.17	1.39
	Internal Control	0.18	0.03	0.80
Inter-Lot Variability	Norovirus G I	0.46	0.21	1.29
	Norovirus G II	0.32	0.10	0.96
	Internal control	0.14	0.02	0.63
Total Variance	Norovirus G I	1.29	1.79	3.66
	Norovirus G II	1.55	2.40	4.95
	Internal control	0.15	0.03	0.69

11.5 Repeatability

To ensure repeatability of the RealStar® Norovirus RT-PCR Kit, specificity and sensitivity are evaluated by analysing established proficiency panels for Norovirus as well as characterized diagnostic samples on a regular basis.

12. Limitations and Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in *in vitro* diagnostic procedure.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay is not to be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the Norovirus genome covered by the tests primers and/or probes may result in failure to detect the presence of the pathogens.
- Noroviruses of Genogroup IV, which have been described to be able to infect humans, get detected by this test in the FAM detection channel.
- As with any diagnostic test, results of the RealStar® Norovirus RT-PCR Kit 2.0 should be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH ISO 13485-certified Quality Management System, each lot of RealStar® Norovirus RT-PCR Kit 2.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Trademarks and Disclaimers

RealStar®, ExtraStar® (altona Diagnostics GmbH); Mx 3005P™ (Stratagene); ABI Prism® (Applied Biosystems); HighPure®, LightCycler® (Roche); Rotor-Gene™, QIAamp® (QIAGEN); VERSANT™ (Siemens); KingFisher® (Thermo Scientific).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The RealStar® Norovirus RT-PCR Kit 2.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Not available in all countries.

16. Explanation of Symbols

In vitro diagnostic medical device

Product number

Batch code

Contains sufficient for “n” tests/reactions (rxns)


Temperature limitation


Version


Use until


Caution


Consult instructions for use


Manufacturer