



EliGene[®] Norovirus LC

REF 90054-LC (for 50 samples)

Kit components:

5 x 150 µl Noro GI LC Mix
5 x 150 µl Noro GII LC Mix
2 x 55 µl Enzyme Mix
1 x 260 µl IC RNA
2 x 50 µl PC DNA Norovirus
1 x Instruction for Use

Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20 °C. Once the reagents are thawed do not freeze them again and store them at 4 °C. Under these conditions they are stable at least for 14 days. MasterMix must be used immediately after preparation. Do not freeze it and store it. Kit must be stored in a dark.

Intended use

EliGene[®] Norovirus LC is intended for the detection and the quantification of Norovirus GI and GII RNA.

Principle of the method

This diagnostic kit is based on Reverse Transcription-quantitative PCR method in one tube. In this kit primers and labeled probes (FAM and HEX) for the detection of Norovirus RNA and for the detection of internal control are used.

Introduction

Noroviruses are a genetically diverse group of single-stranded RNA, non enveloped viruses in the *Caliciviridae* family. The viruses are transmitted by fecally contaminated food or water, by person-to-person contact, and via aerosolization of the virus and subsequent contamination of surfaces. Noroviruses are the most common cause of viral gastroenteritis in humans. Norovirus affects people of all ages. Outbreaks of norovirus infection often occur in closed or semiclosed communities, such as long-term care facilities, overnight camps, hospitals, prisons, dormitories, and cruise ships, where the infection spreads very rapidly either by person-to-person transmission or through contaminated food. Norovirus is rapidly inactivated by either sufficient heating or by chlorine-based disinfectants, but the virus is less susceptible to alcohols and detergents, as it does not have a lipid envelope.

Primary sample collection, handling and storage

Material: feces, water

Recommended RNA isolation procedure: PowerMicrobiome™ RNA Isolation Kit
PowerWater® RNA Isolation Kit

Serum or plasma: According to standard protocol, take the sample of serum or plasma into sterile tubes. Samples must be stored and transported at -20°C.

The kit is optimized for usage of isolation with RNeasy PowerMicrobiome Kit and RNeasy PowerWater Kit (Qiagen) according to standard protocol. **Before the isolation, 5 µl of Internal Control RNA (IC RNA) must be added to the sample after addition of lysis buffer.**

RNA is recommended to be eluted in water for molecular biology. Due to the composition of the elution buffers



of some manufacturers, inhibition of PCR reaction by elution buffer compounds may occur. Elution buffer of isolation kits recommended above can be used with no fear of PCR inhibition. If you intend to use isolation kits from other manufacturers, internal control of amplification (RNA) included in this kit must be added to RNA isolation to ensure that inhibition by elution buffer is excluded.

It is highly recommended use RNA immediately after the isolation for the analysis. You can store RNA at -20°C but concentration of isolated RNA is getting lower in every freezing process and there is a risk of obtaining of false negative results. That is the reason why to carry out RT-PCR immediately after the RNA isolation.

Additional required equipment

- Automatic pipette 5-20 µl and sterile tips with filter DNA RNA free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for RealTime Systems LightCycler® 480, QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific), Rotor-Gene Q (Qiagen) and Real-Time PCR system CFX96 (Bio-Rad). The RT-qPCR for the detection of Norovirus GI and GII RNA utilizes TaqMan technology (FAM and HEX probes) and can be performed on other instruments that can work in FAM and HEX channels.
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves

Configuration of Real Time instrument

- For Norovirus GI and GII detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm)
- For Internal control the probe labeled with HEX is used (exc. 520 nm – em. 548 nm)

LightCycler® 480 (Roche):

Please, use white plates only intended for LightCycler® 480. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.

In option Detection format choose “2 Color Hydrolysis probe”.

Set up the following temperature profile:

Step 1 - Analysis mode “None”, 1 Cycle

55°C	15 min	Ramp rate (4.4°C/s)	Acquisition mode “None”
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Step 2 - Analysis mode “None”, 1 Cycle

95°C	2 min	Ramp rate (4.4°C/s)	Acquisition mode “None”
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Step 2 - Analysis mode “Quantification”, 45 Cycles

95°C	5 s	Ramp rate (4.4°C/s)	Acquisition mode “None”
55°C	15 s	Ramp rate (2.2°C/s)	Acquisition mode “Single”
67°C	15 s	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 3 - Analysis mode “None”, 1 Cycle

40°C	20 s	Ramp rate (2.2°C/s)	Acquisition mode “None”
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The complete temperature profile can be up-loaded from Run Template “EliGene NORO_LC480.ix0”. The Run Template can be imported to the software in menu “Navigator” by clicking to icon “Import” from the CD included



in the kit.

QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific):

Use the Experiment type, "Presence/Absence", Chemistry "TaqMan Probes", and Run Mode "Standard".

Set up the following temperature profile:

Holding stage

55°C 15 min Ramp rate (1.6°C/s)

Holding stage

95°C 2 min Ramp rate (1.6°C/s)

Cycling stage – 45 cycles

95°C 5 s Ramp rate (1.6°C/s)

55°C 15 s Ramp rate (1.6°C/s) Data collection ON

67°C 15 s Ramp rate (1.6°C/s)

Post-Read Stage

40°C 20 s Ramp rate (1.6°C/s)

Collect emission signal at the second step at 55 °C

The complete temperature profile can be up-loaded from Run Template "EliGene NORO_QS3.edt" or "EliGene COVID19 BASIC A RT_QS5.edt". The Run Template can be copied from the CD included in the kit.

RotorGene 6000 or Q (Qiagen):

In the "New Run" window choose "Three Step" run

Choose the appropriate "Rotor Type" and click "Next".

Set up the following temperature profile:

Holding stage

55°C 15 min

Holding stage

95°C 2 min

Cycling stage – 45 cycles

95°C 5 s

55°C 15 s Acquiring in channels "Green" and "Yellow"

67°C 15 s

Holding stage

40°C 20 s

For the Gain optimization in all channels select option "Automatic gain optimization before first acquisition". The complete temperature profile can be up-loaded from Run Template "EliGene NORO_Q-GENE.ret". The Run Template can be copied from the CD included in the kit.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Startup Wizard Create a new Experiment for CFX96 instrument and Create New Protocol.

Set up the following temperature profile:



Step 1	55°C	15 min
Step 2	95°C	2 min
Step 3	95°C	5 s
Step 4	55°C	15 s + Plate Read
Step 5	67°C	15 s
Step 6	<i>GOTO Step 3</i>	<i>44x</i>
Step 7	40°C	20 s
Enter the Sample Volume 20ul		

Collect emission signal at the Step 4 at 55° C.

For filter settings use the “Scan Mode” All Channels but in Plate Manager select for the samples only fluorophores FAM and HEX. Then assign the samples with positions and Targets FAM and HEX as an Unknown sample (Samples) or Standard.”. The complete temperature profile can be up-loaded from Run Template “EliGene NORO_CFX96.prcI”

Reagent preparation

- To avoid the contamination, keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mixed on vortex and shortly spun.
- Add 5 µl of Internal Control RNA (IC RNA) to sample with lysis buffer. Never add Internal Control RNA to isolated RNA before starting PCR!

Preparation of Reaction Mix

1. Take the Noro GI LC Mix and Noro GII LC Mix tube and thaw at room temperature. Immediately after thawing, spin briefly in centrifuge. Prepare the Master Mix by mixing 14 µl Noro GI LC Mix and Noro GII LC Mix with 1 µl Enzyme Mix per reaction and spin briefly.

2. Detection: Add 15 µl of the Master Mix to the amplification tubes or plates and add 5 µl of the isolated RNA sample. Be careful when pipetting the sample to avoid cross-contamination of the samples. The prepared Master Mix should be used within 30 minutes and cannot be reused. Do not freeze prepared Master Mix.

3. Positive Control: Pipette 15 µl of the Master Mix separately into the amplification tube or plate. Then add 5 µl of 5 µl of PC DNA Norovirus. Be careful when pipetting the positive control to avoid contamination of samples.

Use a different micropipette for pipetting only positive controls.

Insert the microtubes or plate into the RealTime PCR instrument and run the program as described in Configuring the RealTime PCR Instrument above.

Result reading

LightCycler[®] 480 (Roche):

In “Sample Editor” menu choose “Abs Quant” workflow.

In menu “Analysis” choose “Abs Quant/2nd Derivative Max” option.

Positive result for *Norovirus GI*: The positive result is characterized by amplification and growth of signal in FAM channel (465-510) of Noro GI Mastermix. In a case of negative results, the amplification will not occur.

Positive result for *Norovirus GII*: The positive result is characterized by amplification and growth of signal in FAM channel (465-510) of Noro GII Mastermix. In a case of negative results, the amplification will not occur.



The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (533-580) in both Noro GI LC and Noro GII LC Mix.

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific):

In "Analyse Settings" choose "Automatic Threshold" and "Automatic Baseline" option and analyze results.

Positive result for *Norovirus GI*: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm) of Noro GI Mastermix. In a case of negative results, the amplification will not occur.

Positive result for *Norovirus GII*: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm) of Noro GII Mastermix. In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in HEX channel (em. 548 nm).

Rotor-Gene Q (Qiagen):

Click to "Analysis" icon in the menu and choose Analysis option "Quantitation". In "Quantitation Analysis" window choose "Dynamic Tube" and "Slope Correct" option.

Positive result for *Norovirus GI*: The positive result is characterized by the growth of fluorescence signal in FAM channel (Green) of Noro GI Mastermix. In a case of negative results, the amplification will not occur.

Positive result for *Norovirus GII*: The positive result is characterized by the growth of fluorescence signal in FAM channel (Green) of Noro GII Mastermix. In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in HEX channel (Yellow).

The values of "Calc. conc." correspond to the quantity of positive result; "Negative" means negative result.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Data Analysis window choose "Quantification". In "Settings" menu choose option "Baseline Threshold" and select "Baseline Cycles" option as "Auto Calculated" and Single "Threshold" option as "Auto Calculated".

In Data Analysis window select a single fluorophore (FAM or HEX) by the clicking the box next to the fluorophore name located under the amplification chart and read the results for individual samples.

Positive result for *Norovirus GI*: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm) of Noro GI Mastermix. In a case of negative results, the amplification will not occur.

Positive result for *Norovirus GII*: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm) of Noro GII Mastermix. In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in HEX channel (em. 548 nm).

Interpretation of results

Negative result:

If the increasing of amplification signal in FAM channel of Noro GI LC mix does not appear before cycle number 40, the result of test should be interpreted as probably Norovirus GI RNA negative or with concentration of Norovirus GI RNA below the detection limit of this kit (10 genomic RNA/reaction). If the increasing of amplification signal in FAM channel of Noro GII LC mix does not appear before cycle number 40, the result of test should be interpreted as probably Norovirus GII RNA negative or with concentration of Norovirus GII RNA below



the detection limit of this kit (10 genomic RNA/reaction). The signal for Internal Control must be positive – see article Quality control. This result does not exclude the occurrence of Norovirus GI or GII infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed Norovirus GI and GII RNA.

Positive result:

Norovirus GI or GII RNA was detected in the sample. The sample is Norovirus GI or GII RNA positive.
Alert: The contamination in laboratory space is also possible. Use separate pipette for mastermixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of RNA and DNA analyses.

Inhibited sample:

In the case that increasing of amplification signal in FAM channel (specific for Norovirus GI and GII) is not observed and Ct value in HEX channel (specific for Internal Control) is higher than 30, it is necessary to repeat the analysis. The best would be to use new RNA prepared by new extraction process.

Control procedure

As an internal control of quality, the Internal Control (IC RNA) for checking the process of RNA isolation, reverse transcription and DNA amplification is used. Positive Control for functional control of Master Mix and as a reference sample is used.

Reference material:

To monitor the all examination process covering RNA isolation and RealTime PCR detection is possible to use reference viral material. The commercial positive material is not available.

Troubleshooting:

1. If there is no amplification of Internal Control, there is some problem in the isolation of RNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Positive Control, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

The sensitivity of EliGene[®] Norovirus LC Kit is 5 genomic RNA (Noroviruses) in reaction mix and it depends on the method of the isolation procedure. The sensitivity of method was verified as follows. There were used the RNA samples of known concentration of genomic RNA. Totally it was tested for three times. The Norovirus detection was 100% successful in all the samples which contain 5 and more genomic RNA/reaction mix.

Analytical sensitivity is 10 copies of Norovirus RNA in reaction mix.

Analytical specificity of method is 100%. Analytical specificity of method was analyzed by comparison of primers and probes with all known RNA and DNA sequences in GenBank database. Analytical specificity was also analyzed by the addition of DNA from HSV1, HSV2, VZV, *E. coli*, *A. niger*, *C. albicans* and RNA from Enterovirus, HCV, RSV1, RSV2, Influenza A, and B to the reaction mix. These DNA or RNA did not give false positive result for Norovirus.

Clinical specificity was tested on human blood and serum samples. 50 samples of different human DNA and RNA isolated from the blood and serum did not give false positive result.

Diagnostic performance characteristics:

Within the frame of testing functional characteristics of the EliGene[®] Norovirus LC kit overall 100 clinical specimens were analyzed. From these specimens 100 blind specimens were verified by reference method (La



Rosa et al. 2010). From these 100 samples 56 samples were Norovirus RNA positive. The EliGene® Norovirus LC kit diagnosed as Norovirus RNA positive all 56 specimens. There were no discrepant results. Totally 44 specimens were right determined by the EliGene® Norovirus LC kit as Norovirus RNA negative. The sensitivity and specificity of the EliGene® Norovirus LC kit is 100%.

Measuring interval

The kit enables the detection of 10^1 - 10^8 of viral DNA molecules in reaction mix.

Internal control of quality

As an internal control of quality the Internal Control for checking the process of RNA isolation together with Positive Control for functional control of mastermix and as a reference sample is used.

Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of RNA). It is strictly recommended to use isolation kits and procedures mentioned above.

Negative result does not exclude the occurrence of Norovirus infection. Results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed Norovirus RNA. The presence of Norovirus RNA of infected persons is dependent on infection phase.

Biological reference intervals

Not applicable information for this kit.

Warning

After mixing MasterMixes must be use immediately. Do not mix components of the kits of different lots.

Warnings and general precautions

This kit is intended for *in vitro* use only.

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.



- Do not use reagents from other manufacturer's kit.
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Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under PCR box or laminar flow box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

The tubes containing Noro GI LC Mix and Noro GII LC Mix are disposable and therefore must be used once only in the preparation of the reaction mixture.

These mixes carry the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P281 Use personal protective equipment as required.

The tubes containing IC RNA are disposable and therefore must be used once only in the preparation of the reaction mixture.

Literature

Bannister BA, Begg NT, Gillespie SH. 2000. Infectious Disease. Blackwell Science, 2th Ed.

Oberste MS, Maher K, Kilpatrick DR, Pallansch MA. 1999. Molecular evolution of the human Noroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification". J. Virol. 73 (3):1941–8



Symbols



Catalog number



Upper limit of temperature



Batch code



Use by (last day of month)



in vitro diagnostic medical device



Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.



Contains sufficient for "N" tests



Attention, consult instructions for use



Manufacturer

Manufacturer

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