Primerdesign™ Ltd

# Norovirus Genogroups 1 and 2

## Norovirus GI capsid protein gene & Norovirus GII RNA dependent RNA polymerase gene

## Genesig® Standard Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

# Introduction to Norovirus Genogroups 1 and 2

Norovirus is known to cause acute gastroenteritis. It is a small (27-38 nm), round, non-enveloped RNA virus belonging to the *Caliciviridae* family and is responsible for over 80% of non-bacterial outbreaks of gastroenteritis in the world. It affects individuals of all ages, with a distinct seasonal link to winter. It has a genome of 7.6 kb that is positive sense and has a single-stranded linear confirmation. It encodes a major structural protein (VP1) of about 58 to 60 kDa and a minor capsid protein (VP2).

Transmission occurs predominantly through ingestion of contaminated water, food and airborne transmission, as well as contact with contaminated surfaces. The ease with which norovirus is transmitted and the low infectious dose required to establish an infection result in extensive outbreaks in numerous environments, such as hospitals, hotels and schools. There is no antiviral drug available to treat this infection, and little is known about its pathogenicity. However, it has been observed that the virus can be taken up by enterocytes where translation of viral nonstructural proteins can occur; it damages and alters intestinal microvilli, leaving them blunt and broadened, thus inhibiting absorption; it causes crypt cell hyperplasia and also leads to apoptosis of enterocyctes.

An incubation period of 24-48 hours is usual. Infection is characterised by the acute onset of nausea, vomiting, abdominal cramps, aching limbs, raised temperature and diarrhoea that generally last for about 48 hours. However, more severe and prolonged infection may be observed in children and the elderly. There are five recognised norovirus genogroups, of which three (GI, GII, and GIV) are known to affect humans and, since 2002, variants of the GII.4 genotype have been the most common cause of norovirus outbreaks. There have been 31 different genotypes identified within the genogroups, with a wide degree of genetic variability present even within each genotype.

# Specificity

The Primerdesign genesig Kit for Norovirus Genogroups 1 (G1) and 2 (G2) (Norovirus) genomes is designed for the *in vitro* quantification of Norovirus genomes. The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology, with over 95% of the NCBI database reference sequences available at the time of design.

The dynamics of genetic variation mean that new sequence information may become available after the initial design. Primerdesign periodically reviews the detection profiles of our kits and, when required, releases new versions.

Our kit for Norovirus GI and GII has been designed for the specific and exclusive *in vitro* quantification of both Norovirus genotypes. Two sets of primers and probe have been designed directed against each genotype, which form distinct genetic linkages. The primers and probe sequences in this kit have 100% homology with over 95% of reference sequences in the NCBI database based on a comprehensive bioinformatics analysis.

If you require further information, or have a specific question about the detection profile of this kit then please send an email to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

# Kit contents

- **Norovirus-specific primer/probe mix (150 reactions BROWN)**
  FAM labelled
- **Norovirus positive control template (for Standard curve RED)**
- **Internal extraction control primer/probe mix (150 reactions BROWN)**
  VIC labelled as standard
- **Internal extraction control RNA (150 reactions BLUE)**
- **Endogenous control primer/probe mix (150 reactions BROWN)**
  FAM labelled
- **RNase/DNase-free water (WHITE)**
  for resuspension of primer/probe mixes
- **Template preparation buffer (YELLOW)**
  for resuspension of internal control template, positive control template and standard curve preparation

# Reagents and equipment to be supplied by the user

**Real-time PCR Instrument**
**Extraction kit**
This kit is recommended for use with the genesig Easy DNA/RNA Extraction kit. However, it is designed to work well with all processes that yield high quality RNA and DNA with minimal PCR inhibitors.

**Oasig™ yophilised OneStep or Precision®PLUS OneStep 2X RT-qPCR Master Mix**
Contains complete OneStep RT-qPCR master mix

**Pipettors and Tips**

**Vortex and centrifuge**

**Thin-walled 0.1 ml PCR reaction tubes**

# Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

If a standard curve dilution series is prepared, this can be stored frozen for an extended period. If you see any degradation in this serial dilution, a fresh standard curve can be prepared from the positive control. Primerdesign does not recommend using the kit after the expiry date stated on the pack.

# Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity (An internal PCR control is supplied to test for nonspecific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative control, replace the template RNA sample with RNase/DNase-free water.

# Dynamic range of test

Under optimal PCR conditions, genesig Norovirus detection kits have very high priming efficiencies of >90% and can detect less than 100 copies of the target template.

# Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primerdesign genesig detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practise the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by US Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by US Patent 5,538,848, owned by The Perkin-Elmer Corporation.

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# Principles of the test

**Real-time PCR**
A Norovirus-specific primer and probe mix is provided, and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the Norovirus cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

**Positive control**
For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template.

This can be used to generate a standard curve of Norovirus copy number / Cq value. Alternatively, the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target Norovirus gene worked properly in that particular experimental scenario. If a negative result is obtained, the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component, which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

**Negative control**
To validate any positive findings, a negative control reaction should be included every time the kit is used. For this reaction, the RNase/DNase-free water should be used instead of the template. A negative result indicates that the reagents have not become contaminated while setting up the run.

**Internal RNA extraction control**
When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control RNA also indicate that PCR inhibitors are not present at a high concentration.

A separate qPCR primer/probe mix is supplied with this kit to detect the exogenous RNA using qPCR. The PCR primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control cDNA does not interfere with detection of the Norovirus target cDNA even when present at a low copy number. The Internal control is detected through the VIC channel and gives a Cq value of 28+/-3 depending on the level of sample dilution.

**Endogenous control**
To confirm extraction of a valid biological template, a primer and probe mix is included to detect an endogenous gene. Detection of the endogenous control is through the FAM channel, and it is NOT, therefore, possible to perform a multiplex with the Norovirus primers. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

# Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

**1. Pulse-spin each tube in a centrifuge before opening.**
This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

**2. Resuspend the primer/probe mixes in the RNase/DNase-free water supplied, according to the table below:**
To ensure complete resuspension, vortex each tube thoroughly.

| Component - resuspend in water | Volume |
|---|---|
| Norovirus primer/probe mix (BROWN) | 165 µl |
| Internal extraction control primer/probe mix (BROWN) | 165 µl |
| Endogenous control primer/probe mix (BROWN) | 165 µl |

**3. Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:**
To ensure complete resuspension, vortex each tube thoroughly.

| Component - resuspend in template preparation buffer | Volume |
|---|---|
| Internal extraction control RNA (BLUE) | 600 µl |
| Norovirus Positive Control Template (RED) * | 500 µl |

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

# RNA extraction

The internal extraction control RNA can be added either to the RNA lysis/extraction buffer or to the RNA sample once it has been resuspended in lysis buffer.

**DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.**

**1. Add 4 µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer per sample.**

**2. Complete RNA extraction according to the manufacturer's protocols.**

# OneStep RT-qPCR detection protocol

**For optimum performance and sensitivity.**
All pipetting steps and experimental plate set up should be performed on ice. After the plate is poured proceed immediately to the OneStep amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artefacts that reduce the sensitivity of detection.

**1. For each RNA sample, prepare a reaction mix according to the table below: Include sufficient reactions for positive and negative controls.**

| Component | Volume |
|---|---|
| oasig OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix | 10 µl |
| Norovirus primer/probe mix (BROWN) | 1 µl |
| Internal extraction control primer/probe mix (BROWN) | 1 µl |
| RNase/DNase-free water (WHITE) | 3 µl |
| **Final Volume** | **15 µl** |

**2. For each RNA sample, prepare an endogenous control reaction according to the table below (optional):**
This control reaction will provide crucial information regarding the quality of the biological sample.

| Component | Volume |
|---|---|
| oasig OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix | 10 µl |
| Endogenous control primer/probe mix (BROWN) | 1 µl |
| RNase/DNase-free water (WHITE) | 4 µl |
| **Final Volume** | **15 µl** |

**3. Pipette 15 µl of these mixes into each well according to your qPCR experimental plate set up.**

**4. Pipette 5 µl of RNA template into each well, according to your experimental plate set up.**
For negative control wells use 5 µl of RNase/DNase-free water. The final volume in each well is 20 µl.

**5. if a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:**

| Component | Volume |
|---|---|
| oasig OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix | 10 µl |
| Norovirus primer/probe mix (BROWN) | 1 µl |
| RNase/DNase-free water (WHITE) | 4 µl |
| **Final Volume** | **15** µl |

**6. Preparation of standard curve dilution series.**

**a. Pipette 90 µl of template preparation buffer into 5 tubes and label 2-6**
**b. Pipette 10 µl of Positive Control Template (RED) into tube 2**
**c. Vortex thoroughly**
**d. Change pipette tip and pipette 10 µl from tube 2 into tube 3**
**e. Vortex thoroughly**

**Repeat steps d and e to complete the dilution series.**

| Standard Curve | Copy Number |
|---|---|
| Tube 1 Positive control (RED) | 2 x $10^5$ per µl |
| Tube 2 | 2 x $10^4$ per µl |
| Tube 3 | 2 x $10^3$ per µl |
| Tube 4 | 2 x $10^2$ per µl |
| Tube 5 | 20 per µl |
| Tube 6 | 2 per µl |

**7. Pipette 5 µl of standard template into each well for the standard curve according to your plate set-up**
The final volume in each well is 20 µl.

# OneStep RT-qPCR Amplification Protocol

Amplification conditions using oasig OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix.

| | Step | Time | Temp |
|---|---|---|---|
| | Reverse Transcription | 10 min | 55 ºC |
| | Enzyme activation | 2 min | 95 ºC |
| Cycling x50 | Denaturation | 10 s | 95 ºC |
| | **DATA COLLECTION *** | 60 s | 60 ºC |

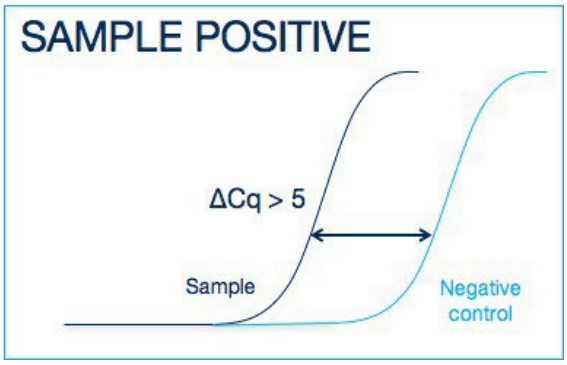
* Fluorogenic data should be collected during this step through the FAM and VIC channels

# Interpretation of results

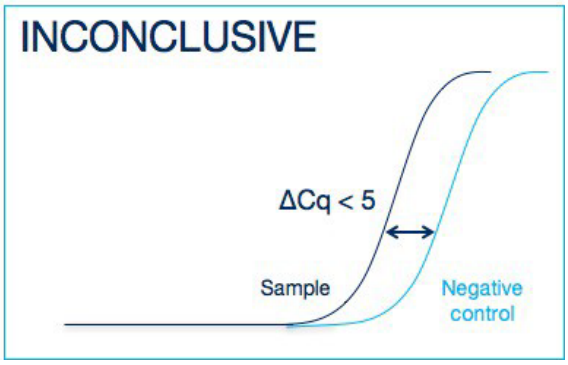
| Target (FAM) | Internal control (VIC) | Positive control | Negative control | Interpretation |
|---|---|---|---|---|
| ≤ 30 | + / - | + | - | POSITIVE QUANTITATIVE RESULT<br>calculate copy number |
| > 30 | + | + | - | POSITIVE QUANTITATIVE RESULT<br>calculate copy number |
| > 30 | - | + | - | POSITIVE QUALITATIVE RESULT<br>Do not report the copy number as this may be due to poor sample extraction |
| - | + | + | - | NEGATIVE RESULT |
| + / - | + / - | + | ≤ 35 | EXPERIMENT FAILED<br>due to test contamination |
| + / - | + / - | + | > 35 | * |
| - | - | + | - | SAMPLE PREPARATION FAILED |
| + / - | + / - | - | + / - | EXPERIMENT FAILED |

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:




If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.




If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

**Internal PCR control**
The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of RNA added to the RT and PCR reaction and the individual machine settings. Cq values of 28±3 is within the normal range. When amplifying a Norovirus sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

**Endogenous control**
The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.