Primerdesign™ Ltd

Human Rotavirus B

Non structural protein 5 (NSP5)

genesig® Standard Kit

150 tests

For general laboratory and research use only

DNA testing

Everything...
Everyone...
Everywhere...

Introduction to Human Rotavirus B

Rotavirus is a genus of double-stranded RNA based viruses of the Reoviridae family. Species within this genus have been named A to G, all cause severe diarrhoea although species A-C are the most common in humans. The linear RNA genome of this species contains 11 segments between 649 and 3538 nucleotides in length. The three layer capsid containing this genome has icosahedral symmetry and is around 80nm in diameter and has channels which extend inward to the core.

The main route of transmission is the faecal-oral route due to contamination of food and water. The virus targets the enterocytes of the intestinal villi, enters the host cell by receptor-mediated endocytosis and replicates in the cytoplasm. After replication and construction of the new viral progeny the cell is lysed resulting in viral release as well as release of intestinal fluid which results in diarrhoea containing viral particles.

After an incubation period of around 2 days, symptoms of Rotavirus infection present with vomiting, fever and diarrhoea that can last over a week. After infection antibodies to the virus can be found in an individual, subsequent infections can occur throughout life but are mainly asymptomatic. Infection is therefore most severe in young children although newborns are thought to have acquired immunity from trans-placental transfer of antibodies. Treatment is usually directed at alleviation of symptoms and mainly involves rehydration.

Specificity

The Primerdesign™ genesig® Kit for Human Rotavirus B (Rotavirus B) genomes is designed for the in vitro quantification of Rotavirus B genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the Rotavirus B genome.
The primers and probe sequences in this kit have 100% homology with a broad range of Rotavirus B sequences 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 e.mail to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

Kit Contents

- **Rotavirus B specific primer/probe mix (150 reactions BROWN)**
  FAM labelled
- **Rotavirus B positive control template (for Standard curve RED)**
- **Rotavirus B RT primer mix (150 reactions GREEN)**
  Required for two step protocol only
- **RNAse/DNAse free water (WHITE)**

Reagents and equipment to be supplied by the user

Real-Time PCR Instrument

RNA extraction kit

This kit is recommended for use with genesig EASY DNA/RNA Extraction kit. However, it is designed to work well with all processes that yield high quality RNA with minimal PCR inhibitors.

oasig™ Lyophilised OneStep or Precision™ OneStep 2x qRT-PCR MasterMix

Contains complete one step qRT-PCR MasterMix

Pipettors and Tips

Vortex and centrifuge

1.5 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. PrimerDesign does not recommend using the kit after the expiry date stated on the pack. Once the lyophilized components have been re-suspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

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. 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® Rotavirus B detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of 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 practice 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 U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks

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Principles of the test

Real-time PCR

A Rotavirus B 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 hybridize to the Rotavirus B cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled 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 real-time PCR platforms.

One Step vs. Two step real-time PCR

When detecting/quantifying the presence of a target with an RNA genome Primerdesign recommend the use of a one step qRT-PCR protocol. One step qRT-PCR combines the reverse transcription and real-time PCR reaction in a simple closed tube protocol. This saves significant bench time but also reduces errors. The sensitivity of a one step protocol is also greater than a two step because the entire biological sample is available to the PCR without dilution. This kit will also work well with a two step approach (Precision™ nanoScript2 reverse transcription kit and PrecisionPLUS™ MasterMix) if required but the use of oasig™ OneStep of Precision™ OneStep MasterMix is the preferred method.

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 Rotavirus B copy number / CT value. (n.b. A new standard curve should be prepared from the positive control template on each occasion rather than freeze/thawing.) 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 Rotavirus B 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 template. A negative result indicates that the reagents have not become contaminated while setting up the run. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources of contamination should first be explored and removed.

Carry-over prevention using UNG (unsuitable for onestep procedure and optional for two step procedure)

Carry over contamination between PCR reactions can be prevented by including uracil-N-glycosylase (UNG) in the reaction mix. Some commercial mastermix preparations contain UNG or alternatively it can be added as a separate component. UNG can only prevent carry over from PCR reactions that include deoxyuridine triphosphate (dUTP) in the original PCR reaction. Primerdesign recommend the application of 0.2U UNG per assay with a 15 minute incubation step at 37°C prior to amplification. The heat-labile UNG is then inactivated during the Taq polymerase activation step (95°C for 10 minutes).

Reconstitution Protocol

To minimize 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. **Reconstitute the kit components in the RNase/DNase-free water supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component	Volume
Pre-PCR pack	
Rotavirus B primer/probe mix (BROWN)	165 µl
Rotavirus B RT primer mix (GREEN)	165 µl
Post-PCR heat-sealed foil	
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.

One Step RT-PCR detection protocol

A one step approach combining the reverse transcription and amplification in a single closed tube is the preferred method. If, however, a two step approach is required see page 11.

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 One Step amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts 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 Precision™ OneStep 2x qRT-PCR MasterMix	10 µl
Rotavirus B primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of this mix into each well according to your real-time PCR experimental plate set up.**

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

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

Component	Volume
oasig™ OneStep or Precision™ OneStep 2x qRT-PCR MasterMix	10 µl
Rotavirus B primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

5. **Preparation of standard curve dilution series.**
n.b. A new standard curve should be prepared from the positive control template on each occasion rather than freeze/thawing.

1) Pipette 900µl of RNAse/DNAse free water into 5 tubes and label 2-6
2) Pipette 100µl of Positive Control Template (RED) into tube 2
3) Vortex thoroughly
4) Change pipette tip and pipette 100µl from tube 2 into tube 3
5) Vortex thoroughly

Repeat steps 4 and 5 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

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

One Step Amplification Protocol

Amplification conditions using oasig™ OneStep 2x qRT-PCR MasterMix.

	Step	Time	Temp
	Reverse Transcription	10 mins	42 °C
	Enzyme activation	2 mins	95 °C
50 Cycles	Denaturation	10 secs	95 °C
	DATA COLLECTION *	60 secs	60 °C

* Fluorogenic data should be collected during this step through the FAM channel

Amplification conditions using Precision™ OneStep 2x RT-qPCR MasterMix.

	Step	Time	Temp
	Reverse Transcription	10 mins	55 °C
	Enzyme activation	8 mins	95 °C
50 Cycles	Denaturation	10 secs	95 °C
	DATA COLLECTION *	60 secs	60 °C

* Fluorogenic data should be collected during this step through the FAM channel

Alternative two step reverse transcription/real-time PCR protocol

Reverse Transcription
If you need to perform separate reverse transcription and amplification (two step Real Time PCR) then we recommend the Primerdesign Precision™ nanoScript2 Reverse Transcription kit. A reverse transcription primer (GREEN) is included and is designed for use with the Precision™ nanoScript2 reverse transcription kit. A protocol for this product is available at www.primerdesign.co.uk

1. **After reverse transcription, prepare a reaction mix according to the table below for each cDNA sample**

Component	Volume
PrecisionPLUS™ 2x qPCR MasterMix	10 µl
Rotavirus B Primer/Probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of this mix into each well according to your real-time PCR experimental plate set up.**

3. **Prepare sample cDNA templates for each of your samples by diluting the RT reaction mix 1:5 in RNAse/DNAse free water.**

4. **Pipette 5µl of cDNA template into each well, according to your experimental plate setup.**
The final volume in each well is 20µl. For negative control wells use 5µl of RNAse/DNAse free water.

Alternative two step amplification protocol

Amplification conditions using PrecisionPLUS™ 2x qPCR MasterMix.

	Step	Time	Temp
	UNG treatment (if required) **	15 mins	37 °C
	Enzyme activation (if required)	2 mins	95 °C
50 Cycles	Denaturation	10s	95 °C
	DATA COLLECTION *	60s	60 °C

* Fluorogenic data should be collected during this step through the FAM channel
** Required if your Mastermix includes UNG to prevent PCR carryover contamination

Interpretation of Results

Target	Negative control	Positive control	Interpretation
+ive	-ive	+ive	**+ive**
-ive	-ive	+ive	**-ive**
-ive	-ive	-ive	**Experiment fail**
+ive	+ive	+ive	**Experiment fail**