Primerdesign

R01276

# Infectious Pancreatic Necrosis Virus

## Kit version: 3

## Target region:
VP2 gene

genesig® Easy RNA Kit

for use on the genesig® q16
50 reactions

GENESIG
Kits by Primerdesign

For general laboratory and research use only

# Product Description

This genesig® Easy qPCR detection kit targets the VP2 gene from infectious pancreatic necrosis virus which is commonly known as IPNV. IPNV is an RNA virus which primarily infects salmonid fish, but may also infect other fish species. Infection can lead to infectious pancreatic necrosis (IPN). The species is divided into the following serogroups: A and B. Serogroup A comprises of nine serotypes and serotype B comprises of one serotype, of which all are predicted to be detected by this qPCR kit.

# Specificity

The kit is designed for the in vitro quantification of infectious pancreatic necrosis virus genomes and to have a broad detection profile. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of last review.

Sequences from the prior five-year period were used for the review. Due to the sequence evolution observed in the IPNV genome over time, this is considered to be an adequate time period for analysis.

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit, please send an email to techsupport@primerdesign.co.uk and our team will answer your question.

# genesig® Easy: at a glance guide

### For each RNA test

| Component | Volume | Lab-in-a-box pipette | |
|---|---|---|---|
| IPNV v3 primer/probe mix | 5 µl | |  |
| Your RNA sample | 5 µl | | |
| oasig® Lyophilised OneStep 2X qPCR Master Mix | 10 µl | | |

### For each positive control

| Component | Volume | Lab-in-a-box pipette | |
|---|---|---|---|
| IPNV v3 primer/probe mix | 5 µl | |  |
| Positive control template | 5 µl | | |
| oasig® Lyophilised OneStep 2X qPCR Master Mix | 10 µl | | |

### For each negative control

| Component | Volume | Lab-in-a-box pipette | |
|---|---|---|---|
| IPNV v3 primer/probe mix | 5 µl | |  |
| RNase/DNase free water | 5 µl | | |
| oasig® Lyophilised OneStep 2X qPCR Master Mix | 10 µl | | |

# Kit Contents

| Quantity | Component | Tube | Cap Colour |
|---|---|---|---|
| 1 | **IPNV v3 primer/probe mix (including internal extraction control primers and probes) (50 reactions)**<br>FAM and VIC labelled as standard |  | **BROWN** |
| 1 | **IPNV v3 positive control template** |  | **RED**<br>(in foil wrapper) |
| 1 | **oasig® OneStep Master Mix** |  | **RED** |
| 1 | **oasig® resuspension buffer**<br>for resuspension of the Master Mix |  | **BLUE** |
| 1 | **Internal extraction control RNA template** |  | **BLUE**<br>(in foil wrapper) |
| 1 | **RNase/DNase free water**<br>for resuspension of primer/probe mixes |  | **WHITE** |
| 2 | **Template preparation buffer**<br>for resuspension of internal control template, positive control template and standard curve preparation |  | **YELLOW** |
| 54 | **genesig® q16 reaction tubes** |  | **N/A** |

# Reagents and equipment to be supplied by the user

### genesig® q16 instrument

### genesig® Easy Extraction Kit

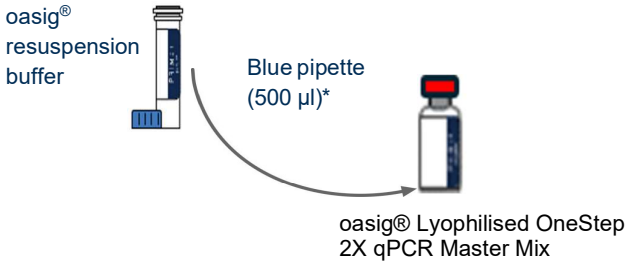
This kit is designed to work well with all processes that yield high quality RNA and DNA but the genesig® Easy extraction method is recommended for ease of use.

### genesig® Lab-In-A-Box

The genesig® Lab-In-A-Box contains all of the pipettes, tips and racks that you will need to use a genesig® Easy kit. Alternatively, if you already have these components and equipment these can be used instead.

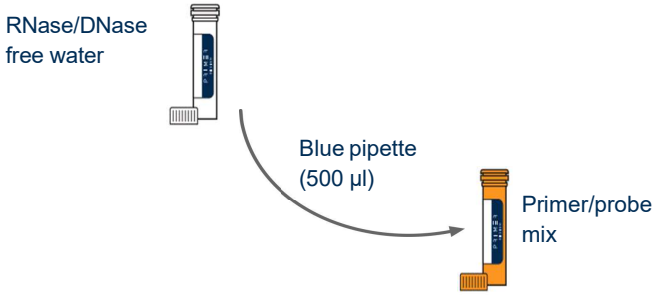
# Step-by-step guide

## 1. Resuspend the test components




Use the blue pipette to transfer 500 µl* of the oasig® resuspension buffer into the tube of oasig® Lyophilised OneStep 2X qPCR Master Mix and mix well by gently swirling.

*Transferring 525 µl of the oasig® resuspension buffer to your oasig® Lyophilised OneStep 2X qPCR Master Mix (instead of the 500 µl recommended above) will enable you to take full advantage of the 50 reactions by accounting for volume losses during pipetting. In order to do so with the Lab-in-a-box fixed volume pipettes use 1x blue, 2x red and 1x grey pipettes to make the total volume. Please be assured that this will not adversely affect the efficiency of the test.



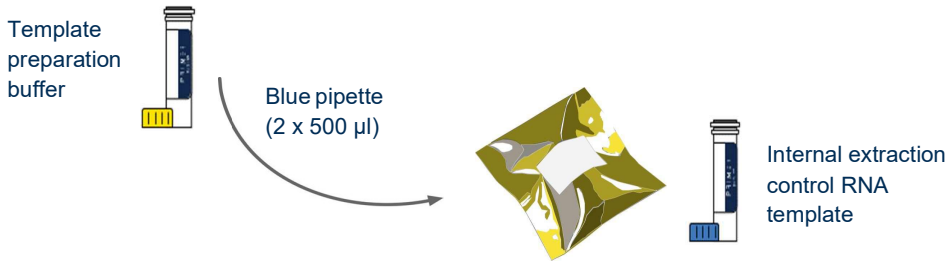

Then use the blue pipette to transfer 500 µl of water into the brown tube labelled IPNV v3 primer/probe mix. Cap and leave for 10 minutes to rehydrate. Then shake tube to mix. A thorough shake is essential to ensure that all components are resuspended. **Failure to mix well can produce poor kit performance.**

Store them in the freezer from hereon.

**Top tip**

- Ensure that the primer/probe mix is mixed thoroughly before each use by shaking and pipetting up and down 10 times.
- Once resuspended do not expose the genesig® Easy kit to temperatures above -20°C for longer than 30 minutes at a time.

# 2. Internal extraction control template




Use the blue pipette to transfer 1000 µl (2 x 500 µl) of template preparation buffer into the Internal Extraction Control RNA template tube. Cap and shake the tube to mix.

The Internal Extraction Control RNA template should be added to your biological sample at the beginning of the RNA extraction process. It is extracted along with the RNA from your target of interest. The q16 will detect the presence of this Internal Extraction Control RNA template at the same time as your target. This is the ideal way to show that your RNA extraction process has been successful.

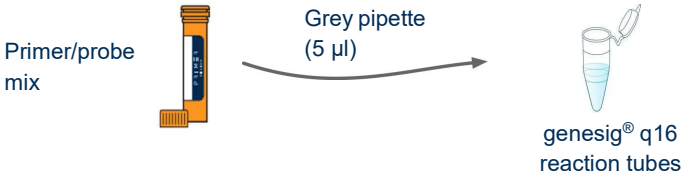
**If you are using an extraction kit:**

Use the red pipette to transfer 10 µl of Internal Extraction Control RNA template to your sample **after** the lysis buffer has been added, then follow the rest of the extraction protocol.

**If using samples that have already been extracted:**

Use the grey pipette to transfer 5 µl of Internal Extraction Control RNA template to your extracted sample.

# 3. Add primer/probe mix to all reaction tubes

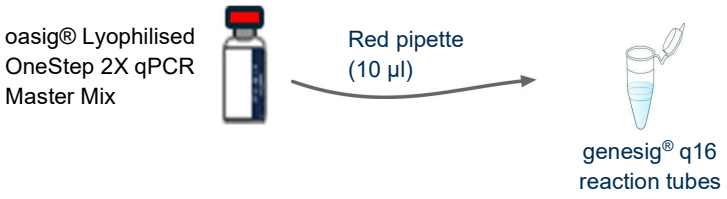



For every reaction to be run, use the grey pipette to add 5 µl of your IPNV v3 primers/probe mix to every genesig® q16 reaction tube.

**Top tip**

- Always pipette the primer/probe mix directly into the bottom of the tube.
- You can label the tube lids to aid your reaction setup but avoid labelling tube sides.

# 4. Add Master Mix to all reaction tubes



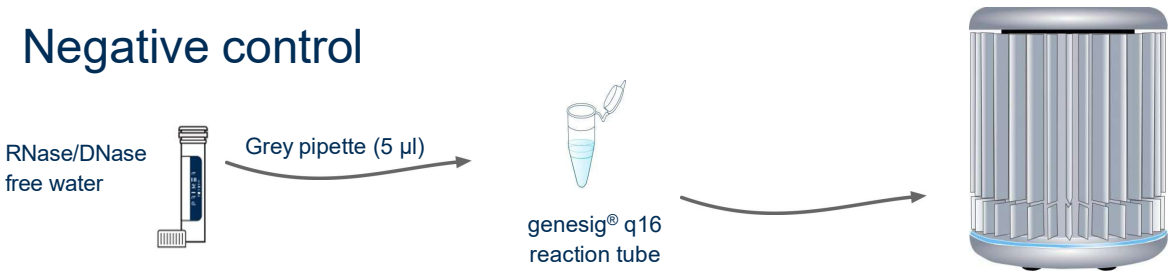

For every reaction to be run, use the red pipette to add 10 µl of the oasig® Lyophilised OneStep 2X qPCR Master Mix to the reaction tubes containing primer/probe mix.

Move swiftly to begin your q16 run, as any delay after the oasig® Lyophilised OneStep 2X qPCR Master Mix has been added can affect the sensitivity of your test.

**Top tip**

- Always add the oasig® Lyophilised OneStep 2X qPCR Master Mix to the side of the tube to reduce the introduction of bubbles.

# 5. Negative control




For each test you will require a negative control. Instead of RNA, water is used. The negative control sample should give a negative test result and thereby prove that any positive samples really are positive, and not tested positive due to contamination.

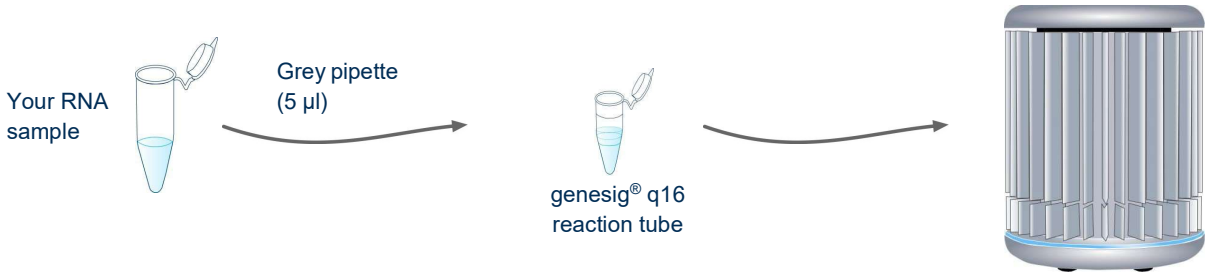
To create a negative control reaction simply use the grey pipette to add 5 µl of the water to the required reaction tube (already containing primer/probe mix and oasig® Lyophilised OneStep 2X qPCR Master Mix). Close this tube after adding the water.

Because some genesig® kit targets are common in the environment you may occasionally see a "late" signal in the negative control. The q16 software will take this into account accordingly.

**Top tip**

- Always add the water to the side of the tube to reduce the introduction of bubbles.

# 6. Set up a test

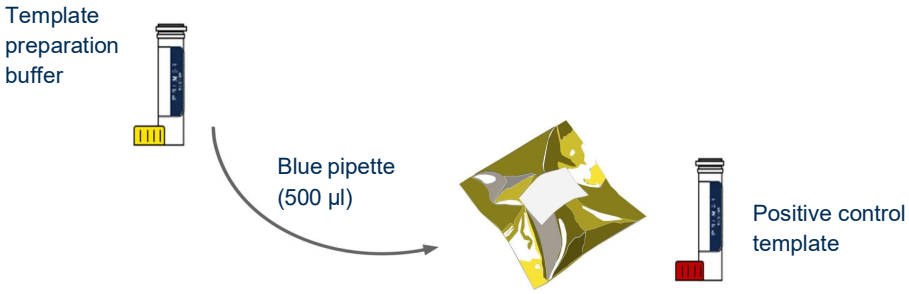



For each sample you wish to analyse, use the grey pipette to add 5 µl of your RNA sample to the required reaction tube (already containing primer/probe mix and oasig® Lyophilised OneStep 2X qPCR Master Mix ). Close these tubes after adding the sample. Always change pipette tips between samples.

**Top tip**

- Always add the RNA sample to the side of the tube to reduce the introduction of bubbles.

# 7. Positive control

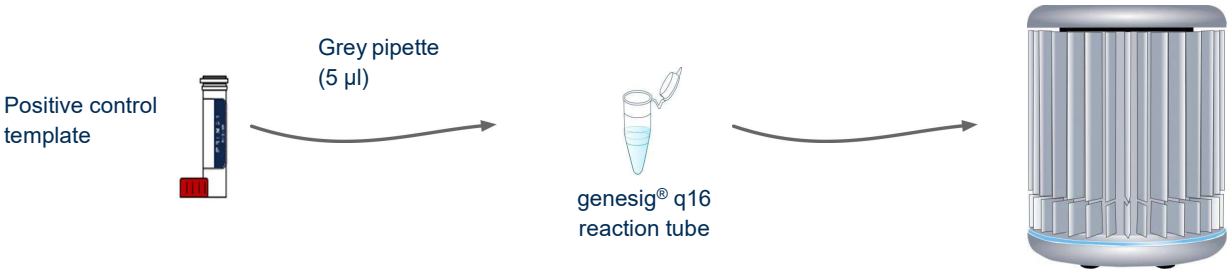



Use the blue pipette to transfer 500 µl of template preparation buffer into the positive control template tube. Cap and shake tube to mix.

Each time you run a test you will require a positive control. It serves two purposes:

1. It will always test positive so it shows that everything is working as it should be.
2. The q16 software knows how many copies of the target are present in the positive control. Therefore, it can automatically compare your sample of interest with the positive control to calculate the amount of target RNA in your sample.

To create a positive control reaction simply use 5 µl of the positive control template instead of your RNA sample and add this to the required reaction tube (already containing primer/probe mix and oasig® Lyophilised OneStep 2X qPCR Master Mix). Close this tube after adding the positive control template.



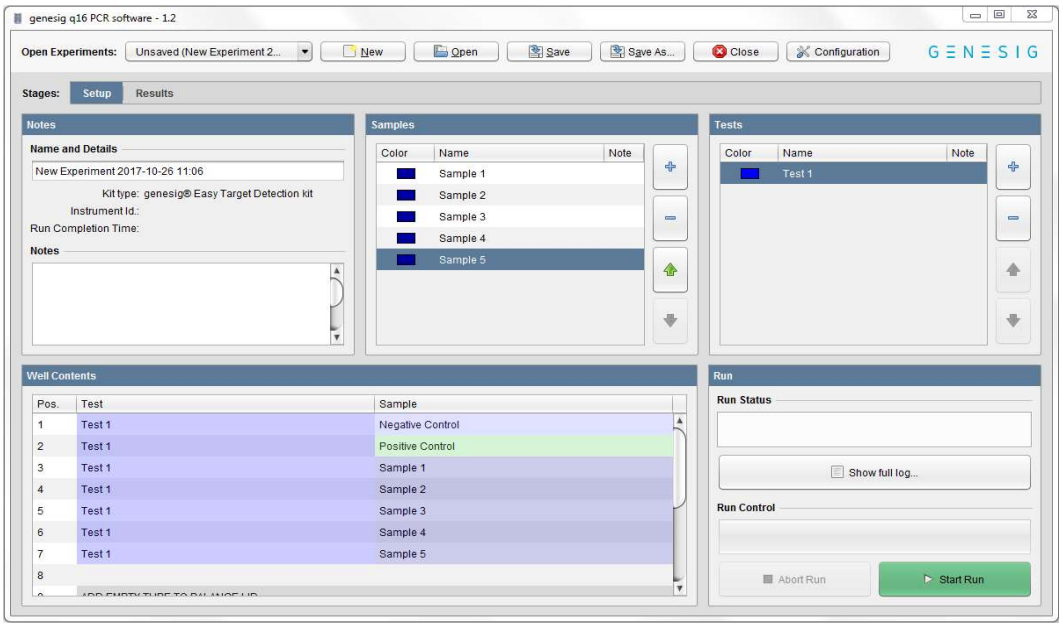

Take great care when setting up your positive control. The positive control template has the potential to give you a false positive signal in your other samples. Set positive controls up last after all other sample tubes are closed. Always change pipette tips between samples. You may even choose to set up positive controls in a separate room.

**Top tip**

- Always add the positive control template to the side of the tube to reduce the introduction of bubbles.

# 8. Running the test

Select the genesig Easy Target Detection Kit module within the software. Place the reaction tubes into the correct positions in your q16 as defined by the software, this may include positioning of empty tubes to ensure that the q16 lid is balanced. The run can then be started.




**Top tip**

- Before loading tubes into the q16, check for bubbles! Flick the bottom of the tubes to remove any bubbles that may have formed during the test setup.
- Apply centrifugal force with a sharp wrist action to ensure all solution is at the bottom of the reaction tube.

# What do my results mean?

Analysis of your data is carried out automatically by the genesig® q16 software. The following information is designed to help you fully understand a result or to troubleshoot:

## “Positive”

### Explanation

Your sample has produced a positive result. Your target of interest is present and you can use the reported quantity.

## “Negative”

### Explanation

Your sample has produced a negative result. The target is not present in your sample.

## “Test contaminated”

### Explanation

The Negative Control should be completely free of any DNA/RNA. If you see this error message it means that at some point during the setup, the Negative Control has been contaminated with DNA/RNA and has given a positive signal. This contamination has invalidated the test. The Positive Control and your test samples are both possible sources of contaminating DNA/RNA. The genesig® q16 reaction tubes from previous runs will also contain very high amounts of DNA so it is important that these are carefully disposed of after the run is completed and NEVER OPENED. It may be the case that your kits have become contaminated which will lead to the same problem occurring repeatedly.

### Solutions

1. Clean your working area using a commercial DNA remover solution to ensure the area is DNA free at the start of your run and re-run the test.
2. If the problem persists, then the kit has become contaminated, and it will have to be discarded and replaced with a new kit. When you open the new kit, run a simple test to show that changing the kit has solved the problem. Prepare a test which includes only the Positive Control, the Negative Control and one ‘mock sample’. For the ‘mock sample’ add internal control template instead of any sample RNA. The result for the Negative Control and the mock sample should be negative indicating that contamination is no longer present.

### Preventive action

An ideal lab set-up has a ‘Clean area’ where the test reagents are prepared and a ‘sample area’ where DNA/RNA samples and the Positive Control template are handled. The best workflow involves setting up all the test components (excluding the positive control template) in the clean area and then moving the tests to the sample area for sample and Positive Control addition. If this method is followed, then the kit components are always kept away from possible sources of contamination. For extra security the Negative Control can be completely prepared and sealed in the clean area. All work areas should be decontaminated regularly with DNA remover.

## “Sample preparation failed”

### Explanation

The test has failed because the quality of the sample was not high enough. The Internal Extraction Control component identifies whether the sample has been prepared correctly and is of suitable quality. This error message means that this quality control test has failed, and the sample quality is not high enough for analysis.

### Solutions

1. Check the sample preparation protocol for any user errors then repeat.
2. Poor quality samples can result from overloading the sample preparation protocol with too much starting material. Try reducing the amount of starting material then repeat.
3. Failing to add the Internal Extraction Control RNA to your sample during the sample preparation protocol can also lead to a reported result of “sample preparation failed”. Ensure that this step has not been overlooked or forgotten. If your samples are derived from an archive store or from a process separate from your genesig® Easy extraction kit; you must add 5 µl of Internal Extraction Control RNA template into each 0.5 ml of your sample to make it suitable for use on the q16.

## “Positive result, poor quality sample”

### Explanation

The test is positive so if you are only interested in obtaining a ‘present or absent’ answer for your sample then your result is reliable. However, the test contains an Internal Extraction Control component that identifies if the sample is of high quality. This quality control test has failed and the sample is therefore not of high enough quality to accurately calculate the exact copy number of RNA present. If you require quantitative information for your sample then proceed with the solutions mentioned above under “Sample preparation failed”.

## “Test failed”

### Explanation

The test has failed because the Positive Control has not worked. The Positive Control is present to show that all aspects of the test are working correctly together. When this control test fails, the test as a whole is invalidated. This finding indicates that a problem has occurred in the reaction set-up part of the experiment and has nothing to do with sample preparation.

### Solutions

1. Check the entire workflow and test set-up to look for any user errors, then repeat the test e.g., have the right colour pipettes and solutions been used with the correct tubes?
2. Ensure the positive and negative controls are inserted into the correct wells of your q16.
3. A component of the test may have ‘gone off’ due to handing errors, incorrect storage or exceeding the shelf life. When you open a new kit, run a simple test to show that changing the kit has solved the problem. Prepare a test which includes only the Positive Control, the Negative Control and one ‘mock sample’. For the ‘mock sample’ add internal control template instead of any sample RNA. If the Positive Control works, the mock sample will now be called as a negative result.

## “Test failed and is contaminated”

### Explanation

The Positive Control is indicating test failure, and the Negative Control is indicating test contamination. Please read the “Test Failed” and “Test contamination” sections of this technical support handbook for a further explanation.

### Solution

For appropriate solutions, read both the “Test failed” and “Test contaminated” sections of this handbook.

# Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon 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. Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

# Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and RNA integrity.

# Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and 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 Primer Design Ltd 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.

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