Primerdesign

R01372

Porcine Reproductive and Respiratory Syndrome Virus

Differentiation of US, EU, and HP strains

Kit version: 1

Target regions

PRRSV-US: ORF6

PRRSV-EU: ORF6

PRRSV-HP: NSP2

genesig®PLEX Kit

100 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Product Description

Porcine reproductive and respiratory syndrome virus (PRRSV) is the pathogen responsible for porcine reproductive and respiratory syndrome, a highly impactful disease in the global swine industry. Infection causes reproductive failure in breeding animals, and respiratory illness in young pigs and piglets.

PRRSV is an RNA virus classified into two major genotypes: PRRSV-1 (PRRSV-EU) and PRRSV-2 (PRRSV-US, also known as PRRSV-NA [North America]).

PRRSV-EU, first identified in Europe, includes strains like the Lelystad virus and is further divided into subtypes, some of which are highly virulent.

PRRSV-US is predominant in North America and Asia, and is known for its genetic diversity across multiple lineages.

Highly Pathogenic PRRSV (PRRSV-HP) is a variant that originated from the PRRSV-US genotype. It is distinguished by a unique deletion in the genome. Infection causes severe clinical signs, including high fever, cyanosis, vomiting, and neurological symptoms, often resulting in high mortality rates among piglets.

Specificity

The genesig®PLEX kit for PRRSV is designed for the in vitro detection of PRRSV-US, PRRSV-EU and PRRSV-HP genotypes. Specifically, the primers will detect 95% of PRRSV-US and PRRSV-EU sequences available on the NCBI database at the time of last review. Due to genomic variation of PRRSV-HP strains and restrictions to maintain exclusivity, the primers will detect 79% of PRRSV-HP sequences.

The PRRSV-US assay may cross-react with rat arterivirus.

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 then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
1	**PRRSV EU/US/HP primer/probe mix (100 reactions)** PRRSV-EU (FAM) PRRSV-HP (ROX) PRRSV-US (Cy5) Internal Extraction Control (VIC)		**BROWN**
1	**PRRSV EU/US/HP positive control template**		**RED** (in silver foil wrapper)
2	**oasig®PLUS OneStep Lyophilised qPCR Master Mix**		**GOLD**
2	**oasig® Master Mix resuspension buffer**		**BLUE**
1	**genesig® Easy RNA internal extraction control**		**BLUE** (in gold foil wrapper)
1	**Template preparation buffer** for resuspension of RNA internal control template and positive control template		**YELLOW**
1	**RNase/DNase free water** for resuspension of the primer/probe mix		**WHITE**

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Must be able to read fluorescence through FAM, ROX, Cy5 and HEX/VIC.

Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

Pipettors and filter tips

Vortex and centrifuge

1.5 ml microtubes

qPCR plates or reaction tubes

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 DNA/RNA integrity. An internal extraction control is provided to test for non-specific PCR inhibitors.

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.

Principles of the test

Real-time PCR

Individual primers and probes designed for each target have been combined into a single reaction and these can be detected through different fluorescent channels as described in the kit contents.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target 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 a positive control, the kit contains a single positive control that contains templates for the 3 targets in the test. The kit positive control will give a PRRSV-EU signal through the FAM channel, a PRRSV-HP signal through the ROX channel, and a PRRSV-US signal through the Cy5 channel. 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 each target are working properly in that particular run. 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 components which would lead to false positive results. This can be achieved by handling this positive control 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 confirm the absence of contamination, a negative control, or No Template Control (NTC) 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.

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 indicates that PCR inhibitors are not present at a high concentration.

The primers and probe necessary to detect the internal extraction control are included in the multiplex primer and probe mix. The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quality and quantity of RNA added to the PCR reaction and the individual machine settings. Cq values of 28 ±3 are within the normal range. When amplifying a target 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.

Resuspension protocol

To minimise the risk of contamination with foreign RNA, we recommend that all pipetting is 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/probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex the tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component - resuspend in water	Volume
Pre-PCR pack	
PRRSV EU/US/HP primer/probe mix (**BROWN**)	110 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
Pre-PCR heat-sealed foil	
genesig® Easy RNA internal extraction control (**BLUE**)	500 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.

4. **Resuspend the lyophilised oasig®PLUS OneStep Master Mix in oasig® resuspension buffer, according to the table below:**

Component - resuspend in oasig® resuspension buffer	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (**GOLD**)	525 µl

RNA extraction

The internal extraction control RNA can be added either to the RNA lysis/extraction buffer or to the 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.**

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 prepared proceed immediately to the 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 all samples, positive and negative controls.

Component	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (**GOLD**)	10 µl
PRRSV EU/US/HP primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (**WHITE**)	4µl
Final Volume	**15 µl**

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

3. **Pipette 5 µl of extracted sample into the sample well according to your experimental plate set-up.**
For negative control wells use 5 µl of RNase/DNase free water (**WHITE**). The final volume for each well is 20 µl.

4. **Pipette 5µl of positive control template into the positive control well, according to your experimental plate set up.**
The positive control contains templates for PRRSV EU/US/HP genotypes. The final volume in each well is 20 µl.

OneStep RT-qPCR Amplification Protocol

Amplification conditions for oasig®PLUS OneStep Lyophilised qPCR Master Mix (**GOLD**)

	Step	Time	Temp
	Reverse transcription	10 mins	55°C
	Enzyme activation	2 min	95°C
Cycling x50	Denaturation	10 secs	95°C
	DATA COLLECTION *	60 secs	60°C

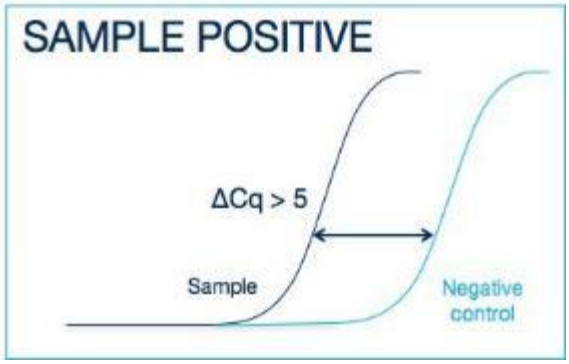
* Fluorogenic data should be collected during this step through the FAM, ROX, Cy5 and VIC channels.

Interpretation of results

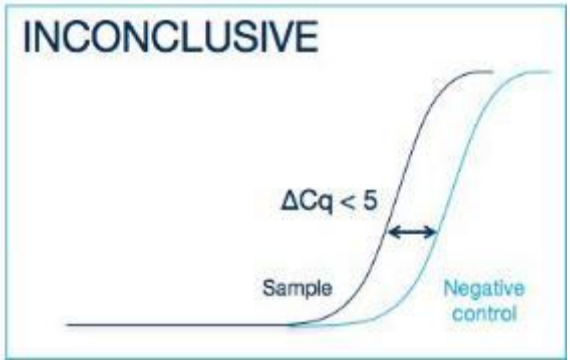
Target	Internal control (VIC)	Positive control	Negative control	Interpretation
FAM+	**+ / -**	**+**	**-**	**PRRSV-EU POSITIVE RESULT**
ROX+	**+ / -**	**+**	**-**	**PRRSV-HP POSITIVE RESULT**
Cy5+	**+ / -**	**+**	**-**	**PRRSV-US POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**
+ / -	**+ / -**	**+**	**≤ 35**	**EXPERIMENT FAILED** 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.

Positive Control

The positive control well should give an amplification plot through the FAM channel (PRRSV-EU), the ROX channel (PRRSV-HP) and Cy5 channel (PRRSV-US). There is no internal control template within the positive control so the VIC channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each target.

No template control (NTC)

The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

RNA Internal extraction control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of nucleic acid in the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a target 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.

Sample data

Presence of the target pathogens is shown by amplification in the channels indicated in the kit contents section. Positive signals indicate positive tests for those targets. It may be possible for samples to contain multiple targets, therefore positive results in more than one of the FAM, ROX and Cy5 channels may be present.

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 in violation of the general GLP guidelines and the manufacturer's recommendations, the right to claim under guarantee is expired.

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