Product Instructions

Porcine Respiratory and Reproduction Syndrome Virus RNA Test Kit

vetproof® Porcine Reproductive and Respiratory Syndrome Virus qPCR Kit

PCR kit for the detection of Porcine Reproductive and Respiratory Syndrome Virus in porcine samples using real-time PCR instruments.

Product No. KIT230156

Reagents for 100 reactions
Store at -20°C

For veterinary use only
For in vitro use only

Contents

1. Product Description

The **vet**proof® Porcine Reproductive and Respiratory Syndrome Virus qPCR Kit (Porcine Reproductive and Respiratory Syndrome Virus RNA Test Kit) will detect the presence of RNA from porcine reproductive and respiratory syndrome virus (PRRSV) genotype I (European, PRRSV EU), II (North American, PRRSV NA) and the highly pathogenic NA strains of PRRSV (PRRSV HP) in extracts from swine samples (whole blood, oral fluid, serum, semen, tissues and nasal swabs and processing fluids). Primers and probes are specific for porcine reproductive and respiratory syndrome virus (PRRSV); each probe is labelled with a specific fluorophore which is detected in a designated channel on the qPCR thermocycler. After extraction of the nucleic acids, samples are added to plates along with the dedicated Reaction Mix. The prepared wells are placed in the qPCR thermocycler for amplification and detection

The **vet**proof® Porcine Reproductive and Respiratory Syndrome Virus qPCR Kit enables the simultaneous detection of:

- PRRSV genotype I (PRRSV EU); detected in the FAM channel.
- PRRSV genotype 2 (PRRSV NA); detected in the HEX channel.
- Highly pathogenic NA strains of PRRSV (PRRSV HP); detected in the ROX channel.
- Process Control; detected in the Cy5 channel.

1.1 Number of Tests

The kit contains reagents for 100 PCR reactions with a final reaction volume of 25 µl each.

1.2 Storage and Stability

Upon receipt, store at -20°C.

WARNING! Read the Safety Data Sheets (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.biochek.com

1.3 Kit Contents

The **vet**proof® Porcine Reproductive and Respiratory Syndrome Virus qPCR Kit contains the following reagents:

1. **Master Mix**, 2 vials, liquid (2 x 750 µl) (yellow cap).
2. **Enzyme Solution,** 2 vials, liquid (2x 55 µl) (red cap)
3. **Process Control,** 2 vials, liquid (2 x 300 µl) (white cap).
4. **Control Template (Positive Control),** 1 vial, liquid (1 x 300 µl) (purple cap).
5. **Negative Control,** 1 vial, liquid (1 x 300 µl) (orange cap)
6. **H_2O PCR-grade**, 1 vial, molecular grade water (1 x 1000 µl) (clear cap).

Note:

- Control Template (Positive Control): Stabilized solution of plasmid DNA containing the target sequences of all targeted pathogens and the Process Control.
- Negative Control: Stabilized solution of plasmid DNA containing only the target sequence of the Process Control.
- Process Control: Stabilized solution of active bacteriophage MS2 (*Emesvirus zinderi*) to be used for monitoring extraction and amplification efficiency of ssRNA viruses.

1.4 Applicability Statement

This kit is compatible with all real-time PCR instruments suitable for detection of FAM, HEX, ROX and Cy5 fluorophores, including the following: LightCycler® 480, LightCycler® 96 (Roche Diagnostics), AriaMx® and Mx3005P® (Agilent Technologies), Applied Biosystems™ 7500 Fast, QuantStudio™ 5 (Thermo Fisher Scientific) and CFX96™ (Bio-Rad Laboratories).

Note: A Color Compensation is necessary and can be supplied by BioChek B.V. for users of the LightCycler® 480 instrument (Color Compensation Set 5; Order No. KIT230011).

1.5 Additional Equipment and Reagents Required

Real-time PCR cycler suitable for the detection of FAM, HEX, ROX and Cy5 labeled probes. Some instruments may have other designations for channels suitable for detection, e.g., VIC instead of HEX or Texas Red (TXR) instead of ROX.

- RNA extraction method and consumables
- Heating block (optional)
- Mini centrifuge (x2)
- Pipettes & disposable filter-tips for volumes of 1 – 20 µl
- Pipettes & disposable filter-tips for volumes of 10 – 100 µl
- Pipettes & disposable filter-tips for volumes of 100 – 1000 µl
- Centrifuge for 8-strip PCR-tubes.
- DNase/RNase free tubes for preparation of reaction mix
- Recommended plates or strips for PCR reaction (suitable for use with your qPCR thermocycler)
- Heat resistant PCR cap strips or sealing foils with appropriate optical quality for fluorophore detection.
- Disposable powder free gloves

1.6 Preparation of Samples

Samples

Swine – oral fluid, serum, whole blood, semen, tissue and nasal swabs.

RNA extraction from samples

Before running the PCR, RNA must be extracted from the sample.

The extraction method chosen to be used with the vetproof® Porcine Reproductive and Respiratory Syndrome Virus qPCR Kit must be suitable for RNA extraction. Recommended are spin column extraction methods or magnetic bead extraction methods (e.g. **vet**proof® MagBead Extraction Kit I – product number KIT230342).

1. Gently mix the process control by inverting 3-5 times (do not vortex) and briefly spin to remove any residues from the lid before adding to the sample. Add 5 µl Process Control to each sample volume used for RNA extraction before starting the RNA extraction procedure.

2. Include an Extraction Control for every RNA extraction procedure. To generate the Extraction Control, add 5 µl Process Control to a volume of PBS (pH 7.0 – 7.6) that corresponds to the typical sample volume of the used extraction kit. Process alongside the field samples (mock purified sample).

- Extracted RNA can be stored at -20°C prior to running the PCR.
- Handle RNA extracts with great care to prevent degradation. Avoid contact with RNase (e.g. from human skin or dirty surfaces) and repeated freeze-thawing.
- It is recommended to verify the performance of the **vet**proof® Porcine Reproductive and Respiratory Syndrome Virus qPCR Kit in combination with the chosen extraction method internally prior to generating results.

2. How to Use this Product

2.1 Good Laboratory Practices for PCR

- Assays must be performed by qualified laboratory personnel only.
- Wear disposable powder free gloves at any stage of running the assay and/or sample preparation. Change gloves when changing work areas or if you suspect that they are contaminated.
- Handle all reagents with care.
- Treat all biological materials as potentially biohazardous, including all field samples.
- Keep the kit components separate from other reagents in the laboratory.
- Never pipette anything by mouth. There must be no eating, drinking, or smoking in areas designated for using kit reagents and handling field samples Use nuclease-free labware (e.g. pipettes, pipette tips, reaction vials).
- To avoid cross-contamination of samples and reagents, use aerosol-preventive pipette tips.
- Physically separate the workplaces for reagent setup (work area 1), DNA/RNA extraction and PCR setup (work area 2), and PCR (work area 3) to minimize the risk of carry-over contamination. Use a PCR hood for all pipetting steps.
- Never move any materials from work area 3 to work area 2 or from work area 2 and 3 to work area 1.
- Change gloves and protective wear when changing work areas to avoid contamination.
- Decontaminate PCR laboratories with bleach or alternative nucleic acid decontaminant and UV light (optional) after testing.

2.2 Procedure

Recommended workflow protocol

1. Extract RNA from the samples before setting up the PCR. This can be done in work area 2 or in another dedicated room not used for any of the steps for PCR setup.
2. Start in work area 1 with reagent preparation.
3. Go to work area 2 for handling extracted samples and assay preparation.
4. Go to work area 3 to run the PCR. Dispose of processed PCR strips in work area 3 after the run.

Note: After preparation the reagent should be immediately transferred to work area 2 and assay setup should be completed in < 1 hour.

Reagent preparation (Work area 1)

1. Defrost the Master Mix at room temperature. The Enzyme Solution is ready to use and does not need to be defrosted.
2. Vortex reagents and briefly spin to remove any residues from the lid.
3. Calculate total volumes of Master Mix and Enzyme Solution required for all reactions (Reaction Mix). Do not forget to include reactions for controls (minimum one positive and one negative), and to compensate for dead volume, + 1 reaction for instance.

Reaction Mix (20 µl)	
Master Mix (yellow Cap)	14 µl
Enzyme solution (red cap)	1 µl
H_2O PCR-grade	5 µl

4. Place the total volume of required Master Mix and Enzyme Solution and H2O into a clean microtube.
5. Gently mix by inverting 3-5 times (do not vortex) and briefly spin to remove any residues from the lid.
6. Take a suitable qPCR plate and add 20 µl of Reaction Mix per PCR well for every sample. The plate does not need to be cooled. Cover the plate and take to work area 2.

Assay Preparation (Work area 2)

1. Add 5 µl of sample RNA extract into each sample well. Record the location of samples on a template.
2. Add 5 µl of Extraction Control into control well. The Extraction Control is contained in the extracted sample set (see 1.6 “Preparation of Samples”).
3. Add 5 µl of Negative Control into control well. This is cross-contamination control.
4. Add 5 µl of Control Template (Positive Control) into control well. It is recommended to seal sample wells before adding the Control Template to avoid undesired cross-contamination.
5. Cover plate with suitable cap strips or a sealing foil.
6. Spin plate for 30-60 seconds at 200-1000 x g

RNA Amplification (Work area 3)

1. Set up the thermocycler and specified thermal cycler program as described in the table below
2. Place plate in qPCR cycler and run the PCR.
3. After the PCR has finished, dispose of the plate in work area 3 in a closed container.

Temperature	Time	No. Of cycles
48 °C	10 min	1
95 °C	3 min	1
95 °C	15 sec	40
60 °C	60 sec	
Data collection (@ 60 °C): FAM = PRRSV EU HEX = PRRSV HP ROX = PRRSV NA Cy5 = Process Control		

NOTE
Alternative dye channels for the reporter dyes:
FAM: no alternative name
HEX: no alternative name
ROX: Texas Red, Red610
Cy5: no alternative name

Note: For some real-time PCR instruments the probe quencher as well as the usage of a passive reference dye must be specified. This kit contains probes with a non-fluorescent “dark” quencher and no passive reference dye.
Note: Do not use “fast mode” on Applied Biosystems™ 7500 Fast, QuantStudio™ 5 or other Thermo Fisher Scientific instruments.

2.3 Validation and Interpretation

After the run has finished, create a new analysis in your qPCR thermocycler software with these settings:

LightCycler® 480	LightCycler® 96	Applied Biosystems™ 7500	Quant Studio™ 5
Abs Quant/Fit Points First Cycle: 5 Last Cycle: 40 Background (6-10)	Abs Quant Settings: Minimal Slope: 0.01 Minimal EPF: 0.100	Auto Baseline *Alternative:* Baseline cycles 3-10	Auto Baseline *Alternative:* Baseline cycles 3-10

CFX96™	AriaMx®	Mx3005P®
Apply Fluorescence Drift Correction Cycles to analyze: 5-40	Background Based Threshold: Cycle Range: 3 thru 7 Sigma Multiplier: 10	Gain Factors: CY5 x1 ROX x1 HEX-JOE x4 FAM x4 Adaptive Baseline

Validation of assay run

The run should be valid, see specific protocol for validation criteria.

If the run was valid the 4 standards can be used to quantify the results.

The 4 results (Cq values) from the standards can be used to calculate the load of an unknown sample.

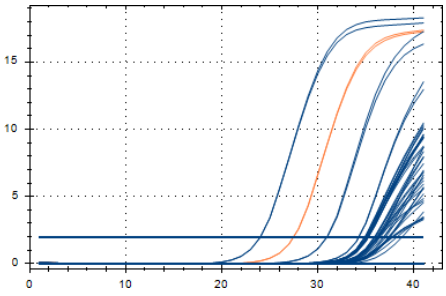
- Check the Cq difference between the dilutions, the dilutions should be +/- 3 Cq apart from each other (10-fold dilution series).
- Make a graph where the exponents (3, 4, 5, and 6) are placed on the X-axis and the Cq number is placed on the Y-axis. Some cycler software can make this standard curve as well.
- Read Cq value from unknown sample on the graph and calculate unknown target load/reaction (x value of the graph, don't forget base number = 10^x)
- Convert this load / reaction to load / ml of sample material (oral fluid, serum, etc) To do this use the input volume before NA extraction together with the elution volume of the NA extraction method and the amount of NA used in the PCR (5 µl).

Note: Other PCR machines can be used, contact BioChek for further information regarding suitable qPCR cyclers.

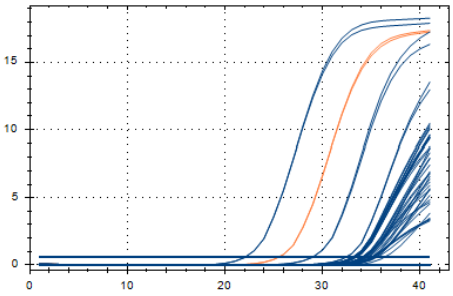
Setting thresholds in the cycler software

This section is only applicable to PCR software analysis using a threshold-based determination of Cq values. Some instruments use a regression-based analysis (e.g., LightCycler® 96), which does not require manual user settings.

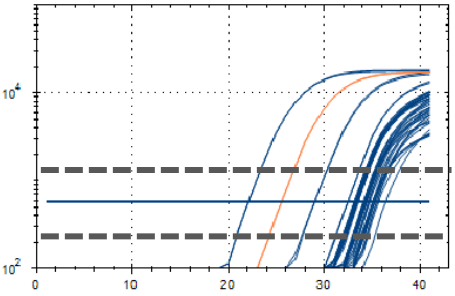
In the analysis section of your PCR instrument software, open the graphical display of the amplification curves. Select all sample-containing wells on the plate, select linear view and select the FAM channel, turn off other channels.



Picture 1



Picture 2



Picture 3

Depending on the number of positive samples, the linear curves should look like the ones in *Picture 1.* To set the threshold, identify the Positive Control reaction (highlighted orange). Look at which cycle the curve transitions into a straight line; in this case, around cycle 27. The threshold is first placed at the point where the curve becomes a straight line. The threshold should then be set halfway between this point and the baseline; see *Picture 2.*

In case highly precise Cq values are desired, the threshold setting can be refined in an optional step. Switch to logarithmic view while keeping the threshold applied. Determine the part of the curve chart where the increase of all curves appears linear; see dashed lines in *Picture 3*. Move the threshold towards the center of this area.

NOTE
Following this procedure will result in reliable positive/negative calls. Certain PCR software may require locking the threshold setting to avoid unwanted automatic recalculation.

Repeat this process for the HEX, ROX and Cy5 channels

Validation of the PCR Run

The following must apply for the PCR run to be valid:

	PRRS EU (FAM) Cq values	**PRRS NA (HEX)** Cq values	**PRRS HP (ROX)** Cq Values	**IC (CY5)** Cq values	**Interpretation**
Positive Control	22-33	22-33	22-33	Not considered	Valid Control
Negative Control	N/A*	N/A*	N/A*	22-33	Valid Control
Extraction Control	N/A*	N/A*	N/A*	<30	Valid Control

** No Cq value*
Note: Repeat the PCR plate in the event of control failure

Validation and interpretation of sample results

Always check the validity of the amplification curves.

PRRSV EU (FAM) Cq values	**PRRSV NA (HEX)** Cq values	**PRRSV HP (ROX)** Cq Values	**Process Control (CY5)** Cq values	**Interpretation**
<40	<40	<40	Not considered	Positive for PRRSV EU, PRRSV NA and PRRSV HP
<40	<40	N/A*	Not considered	Positive for PRRSV EU and PRRSV NA
N/A*	<40	<40	Not considered	Positive for PRRSV NA and PRRSV HP
<40	N/A*	<40	Not considered	Positive for PRRSV EU and PRRSV HP
<40	N/A*	N/A*	Not considered	Positive for PRRSV EU
N/A*	<40	N/A*	Not considered	Positive for PRRSV NA
N/A*	N/A*	<40	Not considered	Positive for PRRSV HP
N/A*	N/A*	N/A*	Cq (Sample) – Cq(Extraction Control) within ± 4 Cq	negative sample Negative for PRRSV EU, PRRSV NA and PRRSV HP
N/A*	N/A*	N/A*	Cq(Sample) – Cq(Extraction Control) outside ± 4 Cq	invalid well**

* No Cq value
** When a sample is negative and the Process Control is out of range, the assay is invalid for this particular sample, and should be repeated with a 1/10 dilution of the extract or a new RNA extract.

3. Supplementary Information

Symbols Glossary

REF	Product Reference Number		Expiry (Expiration) Date
	Kit Size/Reactions		Protect from Moisture
	Store at		Protect from Heat and Direct Sunlight
	Batch		Manufacturer

Quality Control

All products are monitored by our quality control on a batch-to-batch basis. A certificate of analysis (CoA) is available from BioChek B.V.

Ordering Information

This kit and associated products are available from BioChek B.V. For a complete overview and for more information, please visit our website at www.biochek.com.

Trademarks

vetproof® is a trademark of Hygiena Diagnostics GmbH.

Warranty and Disclaimer of Liability

“Limited Warranty” and “Disclaimer of Liability”: BioChek B.V. warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

1. The product is used according to the guidelines and instructions set forth in the product literature.
2. BioChek B.V. does not warrant its product against any and all defects when: the defect is a result of material or workmanship not provided by BioChek B.V.; defects caused by misuse or use contrary to the instructions supplied, or improper storage or handling of the product.
3. All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty.
4. BioChek B.V. does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of BioChek B.V.
5. BioChek B.V. does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death.
6. BioChek B.V. reserves the right to replace or allow credit for any modules returned under this warranty.

Regulatory Disclaimers

For veterinary use only. For in vitro use only.

Regulatory requirements vary by country; this product may not be available in your geographic area. For information on availability, please contact BioChek B.V.

4. Revision Index

Revision A: New document

5. Order Information

For questions regarding ordering or for support:

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Product number	Description
KIT230156	vetproof® Porcine Reproductive and Respiratory Syndrome Virus qPCR Kit
KIT230348	vetproof® Porcine Reproductive and Respiratory Syndrome Virus qPCR Standards
KIT230342	vetproof® MagBead Extraction Kit I

6. Supplier Information

Authorized Representative

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