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virotype® PRRSV PRO + HP RT-PCR Kit Handbook

For detection of RNA from *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV)

REF 100 reactions (Cat. no. VT282405)

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INDICAL BIOSCIENCE

Contents

Kit contents

virotype PRRSV PRO + HP RT-PCR Kit	(100)
Cat. no.	VT282405
Number of reactions	100
RT-PCR Mix (tube with yellow cap) includes enzymes and the exogenous internal control system	2 x 800 µl
Primers/Probes (tube with purple cap)	1 x 210 µl
Positive Control (tube with red cap)	1 x 150 µl
Negative Control (tube with blue cap)	1 x 150 µl
Handbook	1

Intended use

The virotype PRRSV PRO + HP RT-PCR Kit is intended for the simultaneous detection of RNA of PRRSV-1 (European genotype, EU) and PRRSV-2 (North American genotype, NA), and highly pathogenic (HP) strains of the NA genotype from *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV). The RNA can be detected in swine blood, serum, tissue, bronchial swabs, bronchial lavage, saliva, semen samples, and cell culture supernatant samples.

For veterinary use only.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

 Protect from light

 For samples from swine

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of virotype PRRSV PRO + HP RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the virotype PRRSV PRO + HP RT-PCR Kit should be stored at -30°C to -15°C and are stable until the expiration date stated on the label. Avoid repeated thawing and freezing (maximum 3 times), as this may reduce assay sensitivity. Freeze the components in aliquots if they will only be used intermittently.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available from your local sales representative or by Email request to compliance@indical.com.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Introduction

The virotype PRRSV PRO + HP RT-PCR Kit is a highly sensitive solution for the safe and simultaneous detection of PRRSV-1 (EU) and PRRSV-2 (NA) genotypes of *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV) in samples from swine. It can also differentiate Highly Pathogenic (HP) strains of PRRSV-2.

PRRSV infections in swine are very prevalent and continue to cause one of the most economically devastating diseases in swine worldwide leading to respiratory disease in piglets and reproductive failure in pregnant sows.

HP PRRSV, first identified in Asia around 2006, is known for severe clinical signs and high mortality. HP PRRSV strains are widely distributed throughout China and parts of Southeast Asia, including Cambodia, Laos, Myanmar, the Philippines, Thailand, and Vietnam.

Principle

Polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is identified using fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real-time) allows detection of the accumulating product without the need to re-open the reaction tubes afterward.

The virotype PRRSV PRO + HP RT-PCR Kit contains all of the necessary reagents for the detection of PRRSV RNA, including a Positive and Negative Control. With this kit, both reverse transcription and PCR are performed in one reaction tube, reducing the risk of contamination.

The virotype PRRSV PRO + HP RT-PCR Kit uses four specific primer/probe combinations:

- TAMRA fluorescence for RNA of PRRSV-1
- FAM™ fluorescence for RNA of PRRSV-2
- Cy®5 fluorescence for RNA of PRRSV HP strains
- JOE™ fluorescence for the Exogenous Internal Control (IC)

A Positive Control serves to verify the functionality of the reaction mix for the amplification of the PRRSV RNA targets.

RNA extraction

The virotype PRRSV PRO + HP RT-PCR Kit can be used for the detection of PRRSV RNA from swine blood, serum, tissue, bronchial swabs, bronchial lavage, saliva, semen samples, and cell culture supernatant.

Due to the high sensitivity of the test, pools of up to 5 individual samples and collective samples of saliva may be analyzed.

Prior to real-time RT-PCR, viral RNA must be extracted from the starting material.

INDICAL offers a range of validated kits for the extraction of RNA from animal samples.

Extraction based on magnetic beads:

- IndiMag® Pathogen Kit (SP947457)
- IndiMag Pathogen Kit w/o plastics (SP947257)
- IndiMag Pathogen IM2 Cartridge (SP957654C608)
- IndiMag Pathogen IM48 Cartridge (SP947654P608, SP947654P224)
- IndiMag Pathogen KF96 Cartridge (SP947855P196, SP947855P496, SP947855P1696)

Extraction based on spin columns:

- IndiSpin® Pathogen Kit (SP54104, SP54106)
- IndiSpin QIAcube® HT Pathogen Kit (SP54161)

If real-time RT-PCR is not performed immediately after extraction, store the RNA at -20°C or at -80°C for longer storage.

For further information on automated and manual extraction of PRRSV RNA from different sample types, refer to the respective handbook or contact INDICAL Support at support@indical.com.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Pipettes
- Nuclease-free, aerosol-resistant pipette tips with filters
- Sterile 1.5 ml Eppendorf® tubes
- Nuclease-free (RNase/ DNase-free) consumables. Special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR for sensitive identification of viral nucleic acids
- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Real-time cycler with appropriate fluorescent channels
- Appropriate software for chosen real-time cycler
- Appropriate strip tubes and caps or 96-well optical microplate with optical sealing film or cover for chosen real-time cycler
- Benchtop centrifuge for strip tubes or microplate

Important notes

General precautions

The user should always pay attention to the following:

- Use nuclease-free pipette tips with filters.
- Store and extract positive materials (specimens, positive controls and amplicons) separately from all other reagents and add them to the reaction mix in a spatially separated facility.
- Thaw all components on ice before starting the assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test kit past the expiration date.
- Keep samples and controls on ice or in a cooling block during the setup of reactions.

Negative control

At least one negative control reaction should be included in each PCR run, containing all the components of the reaction except for the pathogen template. This enables assessment of contamination in the reaction.

Positive control

When performing PCR on unknown samples, it is recommended to include a positive control reaction in the PCR run containing a sample that is known to include the targeted viral RNA. A positive control serves to prove the functionality of the pathogen assay, e.g., the correct setup of the reaction mix. Use 8 µl of the Positive Control provided with the virotype PRRSV PRO + HP RT-PCR Kit to test for successful amplification of the target.

Exogenous Internal Control

For increased process safety and convenience, the RT-PCR Mix already contains an Exogenous Internal Control assay, including both the target RNA and the corresponding primer/probe set. This allows direct monitoring of the amplification process without adding any separate control material.

Protocol: Real-time RT-PCR for detection of RNA from *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV)

Important points before starting

- Please read „Important notes“ on page 11 before starting.
- Include at least one positive control (Positive Control) and one negative control (Negative Control) per PCR run.
- Before beginning the procedure, read through the protocol and ensure that you are familiar with the operation of the chosen real-time PCR cycler.
- RNA is unstable. Perform the protocol without interruption.

Things to do before starting

- Thaw all reagents on ice and protect from light.
- Before use, spin the reagents briefly.
- Maintain reagents on ice or in a cooling block during PCR setup.

Procedure

1. Before use, mix the RT-PCR Mix by inverting 5 times or until mixed thoroughly, then centrifuge briefly to collect the fluids.
2. Set up the Master Mix according to Table 1 immediately prior to use.

   The Master Mix contains all the components that are required for a PCR reaction except the sample. Set up a Master Mix volume that is 10 % greater than is needed for the total amount of PCR reactions.

   Table 1 lists the required volumes based on the quantity of reactions. Storage of prepared Master Mix is not recommended.

Table 1. Preparation of the Master Mix

	Quantity of reactions		
Component	1	25	50
RT-PCR Mix (yellow cap)	15 µl	375 µl	750 µl
Primers/Probes (purple cap)	2 µl	50 µl	100 µl
Total volume	**17 µl**	**425 µl**	**850 µl**

3. Mix well the prepared Master Mix by inverting 5 times or until mixed thoroughly, then centrifuge briefly to collect the fluids.

4. Pipet 17 µl of the Master Mix into each reaction tube. Then add 8 µl of the sample RNA to give a final reaction volume of 25 µl. (Table 2).

   Include positive and negative control reactions.

   Positive Control: Use 8 µl of the positive control (Positive Control) instead of sample RNA.

   Negative Control: Use 8 µl of the negative control (Negative Control) instead of sample RNA.

Table 2. Preparation of the Reaction Mix

Component	Volume
Master Mix	17 µl
Sample	8 µl
Total volume	**25 µl**

5. Close the reaction tubes with the corresponding caps or seal the plate and **invert vigorously at least 5 times**. Then centrifuge briefly to collect the fluids.

6. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 3.

Table 3. Filter settings for the reporter

Pathogen/ Internal Control	Reporter
PRRSV-1	TAMRA
PRRSV-2	FAM
PRRSV HP	Cy5
Exogenous Internal Control (IC)	JOE
Passive reference[1]	ROX™[1]

1 Internal reference for use with ABI PRISM® Sequence Detection Systems (Applied Biosystems®)

7. Run the real-time RT-PCR protocol according to Table 4.

Table 4. Real-time RT-PCR protocol for PRRSV PRO + HP

Step	Temperature	Time	Number of cycles
Reverse Transcription	50°C	10 min	1
Initial Activation	95°C	2 min	1
2-step cycling			
Denaturation	95°C	5 s	40
Annealing/ Extension*	60°C	30 s	

* Fluorescence data collection, approximate run time 51 min (Applied Biosystems ABI7500 Fast, fast mode)

Data analysis and interpretation

Interpretation of results

For the assay to be valid the Positive Control must give a signal in the TAMRA, FAM, Cy5 and JOE channels with a C_T[1] < 35. The Negative Control must give a JOE fluorescence signal and must not show a signal in the TAMRA, FAM, and Cy5 channels.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 5 on page 22.

The sample is positive for PRRSV-1, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the TAMRA and JOE channels.
- The Positive Control yields a signal in all channels.
- The Negative Control yields a signal in the JOE channel only.

Note that very high concentrations of PRRSV RNA in the sample may lead to a reduced JOE signal or no JOE signal due to competition with the Exogenous Internal Control.

[1] Threshold cycle (C_T) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence

The sample is positive for PRRSV-2, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the FAM and JOE channels.
- The Positive Control yields a signal in all channels.
- The Negative Control yields a signal in the JOE channel only.

Note that very high concentrations of PRRSV RNA in the sample may lead to a reduced JOE signal or no JOE signal due to competition with the Exogenous Internal Control.

The sample is positive for PRRSV HP strain, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the Cy5, FAM and JOE channels.
- The Positive Control yields a signal in all channels.
- The Negative Control yields a signal in the JOE channel only.

Note that very high concentrations of PRRSV RNA in the sample may lead to a reduced JOE signal or no JOE signal due to competition with the Exogenous Internal Control.

The sample is positive for both PRRSV-1 and PRRSV-2, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the TAMRA, FAM, and JOE channels.
- The Positive Control yields a signal in all channels.
- The Negative Control yields a signal in the JOE channel only.

Note that very high concentrations of PRRSV RNA in the sample may lead to a reduced JOE signal or no JOE signal due to competition with the Exogenous Internal Control.

The sample is positive for PRRSV-1, PRRSV-2 and HP, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the TAMRA, FAM, Cy5, and JOE channels.
- The Positive Control yields a signal in all channels.
- The Negative Control yields a signal in the JOE channel only.

Note that very high concentrations of PRRSV RNA in the sample may lead to a reduced JOE signal or no JOE signal due to competition with the Exogenous Internal Control.

The sample is negative for PRRSV, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the JOE channel only.
- The Positive Control yields a signal in all channels.
- The Negative Control yields a signal in the JOE channel only.

A positive JOE signal excludes the possibility of inhibition, as the Exogenous Internal Control was successfully amplified.

The sample results are inconclusive, and the assay is invalid, if the following criteria are met:

- The sample yields a signal in the Cy5 and JOE channels but no signal in the FAM channel.

If no signal is detected in the FAM (PRRSV-2) but in the Cy5 (PRRSV HP) and the JOE (Exogenous Internal Control, IC) channels, the result is inconclusive. This could be the case if a sample is weakly positive (at the detection limit).

We recommend the repetition of the whole test procedure starting with new sample material.

Check that there is a fluorescence signal in all the channels for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be due to incorrect setup of the reaction mix, or incorrect cycling conditions.

The sample results are inconclusive, and the assay is invalid, if the following criteria are met:

- The sample yields no signal in any of the fluorescence channels.

If no signal is detected in the TAMRA (PRRSV-1), FAM (PRRSV-2), Cy5 (PRRSV HP) and the JOE (Exogenous Internal Control, IC) channels, the result is inconclusive. The absence of a signal for the Exogenous Internal Control indicates strong PCR inhibition and/or other malfunctions, e.g., during extraction.

To check for inhibition we recommend 1:5 dilution of the sample RNA in nuclease-free water, to repeat the RNA extraction procedure, or repeat the whole test procedure starting with new sample material.

Check that there is a fluorescence signal in all the channels for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be due to incorrect setup of the reaction mix or incorrect cycling conditions.

Table 5. Results interpretation table*

Sample result	TAMRA (PRRSV-1)	FAM (PRRSV-2)	Cy5 (PRRSV HP)	JOE (IC)
PRRSV-1 positive	X			X
PRRSV-1 strong positive	X			
PRRSV-2 positive		X		X
PRRSV-2 strong positive		X		
PRRSV HP positive		X	X	X
PRRSV HP strong positive		X	X	
PRRSV-1 and -2 positive	X	X		X
PRRSV-1 and -2 strong positive	X	X		
PRRSV-1, PRRSV-2, and HP positive	X	X	X	X
PRRSV-1, PRRSV-2, and HP strong positive	X	X	X	
PRRSV negative				X
Inconclusive			X	X

* Interpretation of sample results can be determined provided positive and negative control reactions are performed. The Positive Control must yield a signal in the TAMRA, FAM, Cy5 and JOE channels. The Negative Control must yield a signal in the JOE channel and no signal in the TAMRA, FAM, and Cy5 channels. For a complete explanation of possible sample results please refer to “Data analysis and interpretation” on page 17.

INDICAL offers a comprehensive range of products to support animal pathogen testing including ELISA kits, qPCR kits and reagents, Master Mixes and nucleic acid extraction chemistry, and instrumentation for automated extraction.

Visit www.indical.com for more information about afosa, bactotype, cador, cattletype, flocktype, IndiField, IndiMag, IndiMix, IndiSpin, pigtype, SVANOVIR, and virotype products.

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Change index

Handbook	Version	Change
HB-2679-EN-001	January 2026	Product launch

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