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virotype® PRRSV EU Vax RT-PCR Kit

Handbook

For detection of RNA from *Porcine Reproductive and Respiratory Syndrome Virus* European vaccines

REF 50 samples (Cat. no. VT282365)

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INDICAL BIOSCIENCE

Contents

Kit contents

virotype PRRSV EU Vax RT-PCR Kit	(50)
Cat. no.	VT282365
Number of reactions	2 x 50
Master Mix A (Porcilis® PRRS, ReproCyc® PRRS EU or Ingelvac PRRSFLEX® EU, tube with **green** cap) includes enzymes, primers, and probes	1 x 850 µl
Master Mix B (UNISTRAIN® PRRS, Suvaxyn® PRRS MLV, tube with **yellow** cap) includes enzymes, primers, and probes	1 x 850 µl
Positive Control (tube with **red** cap)	1 x 150 µl
Negative Control (tube with **blue** cap)	1 x 150 µl
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Intended use

The virotype PRRSV EU Vax RT-PCR Kit is designed for the concurrent detection of European genotype vaccines of the *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV). The vaccines can be detected in extracted RNA from a variety of sample types including serum, tissue, saliva, nasal swabs and processing fluids.

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 EU Vax RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the virotype PRRSV EU Vax 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 3x), 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 EU Vax RT-PCR Kit is designed for the simultaneous detection of European genotype vaccine strains of the *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV) in samples from pigs. In the swine industry PRRSV remains a significant pathogen causing respiratory disease in piglets and reproductive failure in pregnant sows.

Detection of European PRRSV vaccine strains (PRRSV-1) are crucial for monitoring vaccination programs and distinguishing between wild-type and vaccine strains. A screening PCR like virotype PRRSV 2.0 RT-PCR Kit (VT282325) indicates the presence of EU genotype, but an additional PCR is required to determine if it is a vaccine strain.

The kit allows for simultaneous identification of all major European PRRSV vaccines, including in Master Mix A Porcilis® PRRS (MSD Animal Health, Intervet Deutschland GmbH, Germany) and ReproCyc® PRRS EU or Ingelvac PRRSFLEX® EU (Boehringer Ingelheim Vetmedica GmbH, Germany) and in Master Mix B UNISTRAIN® PRRS (Laboratorios Hipra S.A., Spain), and Suvaxyn® PRRS MLV (Zoetis Belgium S.A., Belgium), which are commonly used for active immunization of pigs against PRRSV infection.

These live-attenuated vaccines are based on PRRSV-1 strains, which are the most common in Western and Central Europe but can be found globally.

The kit can be used with extracted RNA from a variety of sample types including serum, tissue, saliva, nasal swabs and processing fluids.

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 EU Vax RT-PCR Kit contains all of the necessary reagents for the detection of PRRSV vaccine 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.

An internal control excludes the possibility of false-negative results.

The virotype PRRSV EU Vax RT-PCR Kit uses three specific primer/probe combinations per Master Mix:

Master Mix A:

- FAM™ fluorescence for RNA of Porcilis® PRRS
- Cy®5 fluorescence for RNA of ReproCyc® PRRS EU or Ingelvac PRRSFLEX® EU
- JOE™ fluorescence for the exogenous Internal Control RNA

Master Mix B:

- FAM™ fluorescence for RNA of UNISTRAIN® PRRS
- Cy®5 fluorescence for RNA of Suvaxyn® PRRS MLV
- JOE™ fluorescence for the exogenous Internal Control RNA

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 EU Vax RT-PCR Kit can be used for the simultaneous detection of PRRSV vaccines in extracted RNA from a variety of sample types including serum, tissue, saliva, nasal swabs and processing fluids.

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
- Benchtop centrifuge for strip tubes or microplate
- 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

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. Avoid repeated thawing and freezing (**maximum 3x**), as this may reduce assay sensitivity.
- 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 EU Vax RT-PCR Kit to test for successful amplification of the target.

Internal control

For increased process safety and convenience, an internal control assay is included in the form of an additional primer/probe set in the master mix. This allows amplification to be monitored.

Protocol: Real-time RT-PCR for detection of RNA from *Porcine Reproductive and Respiratory Syndrome Virus* European vaccines

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. Avoid repeated thawing and freezing (**maximum 3x**), as this may reduce assay sensitivity.
- Before use, spin the reagents briefly.
- Maintain reagents on ice or in a cooling block during PCR setup.

Procedure

1. Before use, mix the Master Mix by inverting 5 times or until mixed thoroughly, then centrifuge briefly to collect the fluids.
2. Pipet 17 µl of the Master Mix into each reaction tube. Then add 8 µl of the sample RNA (Table 1).

   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 1. Preparation of reaction mix

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

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

4. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2.

Table 2. Filter settings for the reporter

Pathogen/ Internal Control Master Mix A	Pathogen/ Internal Control Master Mix B	Reporter
Porcilis® PRRS	UNISTRAIN® PRRS	FAM
ReproCyc® PRRS EU or Ingelvac PRRSFLEX® EU	Suvaxyn® PRRS MLV	Cy5
Exogenous Internal Control (IC)	Exogenous Internal Control (IC)	JOE[1]/HEX™[1]
Passive reference[2]	Passive reference[2]	ROX™

1 Use the option appropriate for your thermal cycler.

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

5. Run the real-time PCR protocol according to Table 3.

Table 3. Real-time RT-PCR protocol for PRRSV EU Vax

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 66 min (ABI 7500 Real-Time PCR System, Thermo Fisher Scientific, Inc.)

Data analysis and interpretation

Interpretation of results

For the assay to be valid the Positive Control must give a signal in the FAM, Cy5 and JOE/ HEX channels with a C_T[1] < 35. The Negative Control must yield a signal in the JOE/HEX channel and no signal in the FAM and Cy5 channels.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 4 on page 20 and Table 5 on page 24.

[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

Master Mix A

The sample is positive for Porcilis® PRRS vaccine, and the assay is valid, if the following criteria are met:

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

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 Internal Control.

The sample is positive for ReproCyc® PRRS EU or Ingelvac PRRSFLEX® EU, and the assay is valid, if the following criteria are met:

- The sample yields a signal in both the Cy5 and JOE/ HEX channels.
- The Positive Control yields a signal in all channels.
- The Negative Control yields a signal in the JOE/ HEX channel.

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 Internal Control.

The sample is negative for Porcilis® PRRS and ReproCyc® PRRS EU / Ingelvac PRRSFLEX® EU, and the assay is valid, if the following criteria are met:

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

A positive JOE signal means that amplification was successful as the Internal Control is amplified.

Note:

A positive PCR test for a vaccine strain cannot definitively rule out the possibility of a recombinant (mixed) strain between various vaccine strains or vaccine strains and field strains. This limitation arises because PCR tests typically target specific genomic regions, which cannot capture recombination events. Even partial sequencing of the PRRSV genome can only indicate probabilities of strain assignment (vaccine, field or recombinant strain). Full-length genome sequencing and advanced bioinformatics analyses are often necessary to accurately detect and characterize recombinant PRRSV strains.

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 FAM, Cy5 and the JOE/ HEX channels, the result is inconclusive. The absence of a signal for the exogenous Internal Control indicates strong PCR inhibition and/or other malfunctions.

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 4. Results interpretation table for using Master Mix A*

Sample result	FAM Porcilis®	Cy5 ReproCyc®/ Ingelvac	JOE IC
Porcilis® PRRS **positive**	X		X
Porcilis® PRRS **strong positive**	X		(X)
ReproCyc® PRRS EU / Ingelvac PRRSFLEX® EU **positive**		X	X
ReproCyc® PRRS EU / Ingelvac PRRSFLEX® EU **strong positive**		X	(X)
Porcilis® PRRS and ReproCyc® PRRS EU / Ingelvac PRRSFLEX® EU **negative**			X
Inconclusive			

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

Master Mix B

The sample is positive for UNISTRAIN® PRRS, and the assay is valid, if the following criteria are met:

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

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 Internal Control.

The sample is positive for Suvaxyn® PRRS MLV, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the Cy5 and JOE/ HEX channels.
- The Positive Control yields a signal in all channels.
- The Negative Control yields a signal in the JOE/ HEX channel.

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 Internal Control.

The sample is negative for UNISTRAIN® PRRS and Suvaxyn® PRRS MLV, and the assay is valid, if the following criteria are met:

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

A positive JOE signal means that amplification was successful as the Internal Control is amplified.

Note:

A positive PCR test for a vaccine strain cannot definitively rule out the possibility of a recombinant (mixed) strain between various vaccine strains or vaccine strains and field strains. This limitation arises because PCR tests typically target specific genomic regions, which cannot capture recombination events. Even partial sequencing of the PRRSV genome can only indicate probabilities of strain assignment (vaccine, field or recombinant strain). Full-length genome sequencing and advanced bioinformatics analyses are often necessary to accurately detect and characterize recombinant PRRSV strains.

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 FAM, Cy5 and the JOE/ HEX channels, the result is inconclusive. The absence of a signal for the exogenous Internal Control indicates strong PCR inhibition and/or other malfunctions.

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 for using Master Mix B*

Sample result	FAM UNISTRAIN®	Cy5 Suvaxyn®	JOE IC
UNISTRAIN® PRRS **positive**	X		X
UNISTRAIN® PRRS **strong positive**	X		(X)
Suvaxyn® PRRS MLV **positive**		X	X
Suvaxyn® PRRS MLV **strong positive**		X	(X)
UNISTRAIN® PRRS and Suvaxyn® PRRS MLV **negative**			X
Inconclusive			

* Interpretation of sample results can be determined provided positive and negative control reactions are performed. The Positive Control must yield a signal in the FAM, Cy5 and JOE/HEX channels. The Negative Control must yield a signal in the JOE/HEX channel and no signal in the FAM and Cy5 channels.
For a complete explanation of possible sample results please refer to “Data analysis and interpretation” for Master Mix B on page 21.

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.

For up-to-date licensing information and product-specific disclaimers, see the respective product handbook or user manual.

Notes

Notes

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Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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Handbook	Version	Change
HB-2670-EN-001	August 2025	Product launch

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