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VetMAX™ Peste des Petits Ruminants Virus Kit

TaqMan® real-time RT-PCR for detection of PPRV (Peste des Petits Ruminants Virus)

Catalog Number PPRP50

Doc. Part No. 100020417 **Pub. No.** MAN0008851 **Rev.** B.0

Technology	Species	Nucleic acid isolated from matrices	Test type
Real-time RT-PCR (RNA) - Duplex - Endogenous IPC	Small ruminants (sheep, goat)	EDTA blood Organs (lung, spleen, intestine, lymph nodes) Swabs (ocular and nasal)	Individual

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

⚠ **WARNING! POTENTIAL BIOHAZARD.** Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Information about the product

Description of the product

The **Applied Biosystems™ VetMAX™ Peste des Petits Ruminants Virus Kit** is a molecular diagnostic tool for detecting PPRV (Peste des Petits Ruminants Virus) in small ruminants (sheep and goats) by real-time RT-PCR.

The Peste des Petits Ruminants Virus is a highly contagious disease (in List A of the OIE) caused by an RNA virus of the *Morbillivirus* genus that affects small ruminants, both domestic and wild. It is a disease characterized by rapid spreading, high morbidity and some cases of mortality.

Each RNA sample obtained after extraction is analyzed in a single well: the same well is used to specifically detect the viral RNA of PPRV and to detect an IPC (Internal Positive Control). A positive IPC reflects both the efficiency of extraction and the absence of inhibitor in the samples.

It can be used with viral RNA extracted from **blood collected in EDTA tubes**, **organs** and **tissues** (lungs, spleen, intestine, lymph nodes), and **swabs** (ocular and nasal).

Complete protocols for viral RNA extraction from these matrices are available upon request from Technical Support.

Kit contents and storage

The **VetMAX™ Peste des Petits Ruminants Virus Kit** contains components that can be used for detecting both PPRV and an IPC. Upon receipt, the whole kit should be stored between **–30°C and –10°C**. After initial use of a component, store it according to the following recommendations:

Component	Description	Volume (50 reactions)	Storage	
			Upon receipt	After initial use
3 - Mix PPR (Green tube)	Mix for TaqMan® RT-PCR. Contains: • The detection system for the PPRV target, including a TaqMan® probe labeled **FAM™ – TAMRA™**. • The detection system for the IPC, including a TaqMan® probe labeled **VIC™ – NFQ** (Non-Fluorescent Quencher). • Buffer, reverse transcriptase and real-time PCR enzyme.	2 × 500 µL	–30°C to –10°C	–30°C to –10°C
4a - EPC PPR (Brown tube)	**External Positive Control:** PPRV positive control. It uses nucleic acid **already extracted** for amplification during the real-time RT-PCR.	90 µL	–30°C to –10°C	–30°C to –10°C

Extraction and amplification controls

The **VetMAX™ Peste des Petits Ruminants Virus Kit** contains one control, enabling validation of the amplification of the viral RNA.

4a - EPC PPR: PPRV positive control

A positive control **already extracted** for amplification during the real-time RT-PCR.

A positive result within the specified C_t range validates the amplification of the PPRV target by real-time RT-PCR.

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Validation of nucleic acid extraction for each sample is done by detection of an **endogenous IPC** (Internal Positive Control), **present in each sample**.

A positive result with a compliant value in a sample validates the extraction of this sample, whether positive or negative for the target pathogen, thus eliminating false negatives and verifying the effect of the inhibitors.

We recommend including two negative controls to confirm correct analysis:

NCS: negative extraction control

This control consists of reagents used in the extraction without addition of the sample (the sample volume can be replaced by the buffer used in the sample preparation or by DNase/RNase-free water) that undergoes the same treatment as the samples, namely nucleic acid extraction and real-time RT-PCR.

A negative result for PPRV and the endogenous IPC confirms the absence of contamination during the extraction and the real-time RT-PCR.

NC: negative amplification control

This is the amplification mixture deposited on the plate during preparation of the real-time RT-PCR, completed with 5 µL of DNase/RNase-free water to adjust the solution to 25 µL.

A negative result for PPRV and the IPC confirms the absence of contamination during real-time RT-PCR reaction preparation.

Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**.

- Precision micropipettes (range of 1 µL to 1000 µL) with DNase/RNase-free filtered tips
- DNase/RNase-free water
- 1X TE buffer
- 1X PBS buffer
- A real-time PCR thermal cycler capable of detecting the following fluorophores:
  - FAM™ (emission maximum: λ515 nm)
  - VIC™ (emission maximum: λ554 nm)
- Optical-quality consumables compatible with the thermal cycler used:
  - PCR 96-well plates, PCR strips (8 or 12 wells), microtubes or capillaries
  - Suitable plate covers or caps for capping

Analysis procedure

The real-time RT-PCR reaction volume is 25 µL:

- **3 - Mix PPR:** 20 µL per reaction
- **Extracted RNA:** 5 µL per reaction

Extraction of viral RNA

RNA must be extracted from the samples prior to real-time RT-PCR analysis.

NOTE: For information about extraction methods that are compatible with and validated for the VetMAX™ Peste des Petits Ruminants Virus Kit, please contact Technical Support.

Preparation of the real-time RT-PCR

1. Create an analysis plan for distribution of the mixes and samples. Keep the positive control (EPC) away from the other samples if possible.
2. Thaw the tube of **3 - Mix PPR** at **2°C to 8°C on ice** or on a refrigerated rack.
3. Homogenize the tube of **3 - Mix PPR** by shaking gently, then centrifuge briefly.
4. Add **20 µL of 3 - Mix PPR** to each well of the PCR plate, PCR strip or capillary used.
5. Add RNA from the samples and controls to the reaction mix, according to the following preset analysis plan:

Type of analysis	Component	Sample volume
Sample for analysis	RNA extracted from sample	5 µL
Positive amplification control	**4a - EPC PPR**	5 µL
Negative extraction control (NCS)	Extracted NCS	5 µL
Negative amplification control (NC)	DNase/RNase-free water	5 µL

6. Cover the PCR plate, PCR strips or capillaries with an adhesive plate cover or suitable caps.

Amplification by real-time RT-PCR

1. Create the following detectors on the thermal cycler:

	Reporter	Quencher
PPR	FAM™	TAMRA™[1]
IPC PPR	VIC™	NFQ (Non-Fluorescent Quencher)
Passive reference: ROX™[1]		

[1] The fluorophores TAMRA™ and ROX™ are required for real-time RT-PCR analysis if the thermal cycler is capable of detecting them. For other thermal cyclers, the absence of detection of these fluorophores does not affect the real-time RT-PCR analysis.

2. Assign the **PPR** detector and the **IPC PPR** detector to each sample well used in the analysis.
3. Set up the following real-time RT-PCR program for the analysis:

	Step repetitions	Temperature	Duration
Step 1	×1	45°C	10 minutes
Step 2	×1	95°C	10 minutes
Step 3	×45	95°C	15 seconds
		60°C[1]	1 minute

[1] Collection of fluorescence data during the 60°C – 1 minute stage.

4. Place the PCR plate, the PCR strips or the capillaries in the thermal cycler and run the real-time RT-PCR.

Interpretation of results

Analysis of the raw data

Refer to the recommendations of the thermal cycler manufacturer for the analysis of the raw data.

1. Position the threshold limits separately for each target of the real-time RT-PCR.
2. For each detector, interpret the results according to the sample C_t values obtained as recommended below.

Validation

The test is validated if the following criteria are met:

	PPR detector	IPC PPR detector	Validation
EPC PPR	$C_t = C_{t\,QC}$ PPR of **4a - EPC PPR** ± 3C_t[1]	C_t < 45 or C_t > 45[2]	RT-PCR validated
NCS	C_t > 45	C_t > 45	Extraction validated
NC	C_t > 45	C_t > 45	PCR reagents validated

[1] Refer to the values listed in section 2.1 "EPC" of the Certificate of Analysis of the lot used for the test.
[2] The IPC value in the EPC should not be used for test validation.

Interpretation of results

For each sample analyzed, the results should be interpreted as shown below:

PPR detector	IPC PPR detector	Interpretation
C_t < 45	C_t < 45 or C_t > 45	PPRV detected
C_t > 45	C_t < 45	PPRV not detected
C_t > 45	C_t > 45	Not validated[1]

[1] The sample will be returned as not validated due to the negative IPC.

Procedure for handling non-validated samples

1. Dilute the non-validated sample RNA at a 1:5 dilution in 1X TE buffer.
2. Perform RT-PCR analysis on 5 µL of this dilution.
3. If the diluted RNA is positive for PPRV or negative for PPRV with a compliant IPC result, the obtained result is then validated.
4. If the diluted RNA is negative for PPRV with a non-compliant IPC result, the obtained result is still not validated. In this case, repeat the nucleic acid extraction using the sample pre-diluted 1:2 in 1X PBS buffer before extraction.
5. If the result is still not validated, repeat the analysis on a new sample.

Documentation and support

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)
  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision history of Pub. No. MAN0008851 (English)

Revision	Date	Description
B.0	23 May 2017	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	24 March 2014	Baseline for revision history

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