Certified management system

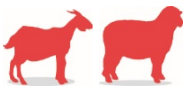
ID Gene™

Peste des Petits Ruminants Duplex

Ref: IDPPR-50 / IDPPR-100
50 / 100 tests

Real-time RT-PCR assay for the qualitative detection of PPR virus
Suitable samples: Caprine and Ovine whole blood, swabs (oral, nasal, ocular or rectal), organ or tissue.

In-vitro Use



IDvet Genetics, 310, rue Louis Pasteur – Grabels - FRANCE
Tel:+ 33 (0)4 67 41 49 33 - Fax: + 33 (0)4 67 45 36 95
www.id-vet.com - E-mail: info@id-vet.com

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General information

▪ Characteristics

ID Gene™ Peste des Petits Ruminants (IDPPR) kit is a real-time RT-PCR kit that amplifies a target sequence in Peste des Petits Ruminants (PPRV) viral genome.

This kit is a qualitative duplex test. It simultaneously amplifies target RNA and an endogenous internal control.

The kit contains a target positive control (TPC-PPR) which is to be extracted in the same manner as the samples to validate the extraction and amplification of the target

This kit can be used to test caprine and ovine whole blood collected in EDTA, swabs and organ or tissue.

▪ Kit composition and storage conditions

The IDPPR kit contains the reagents shown below:

Reference	*Component*	*Volume*	*Description*
TPC-PPR	Target Positive Control	550 µl 1 vial	Inactivated PPRV vaccine, diluted in a virus-free whole blood, freeze-dried and calibrated at between 1 and 100 times the method detection limit (MDL). Freeze-dried pellet to be reconstituted in **550 µl** distilled or Nuclease-free water.
ARM-PPR	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mixture containing the reverse transcriptase, Taq polymerase and oligonucleotides for amplification and detection of PPRV and of the endogenous non-target positive control.

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) in order to avoid multiple freeze/thaw cycles (not more than 3 cycles).

▪ Material required but not provided in the kit

All material used should be of suitable quality for molecular biology.

Amplification Instrument:

- Real-time thermal cycler with channels capable of reading the following fluorophores: FAM, HEX or VIC and Cy5.

  Examples of compatible thermal cyclers: CFX96™, Chromo4™ (Biorad), LC®480 I, LC®480 II, LC®96 Roche, AB® 7500 and Rotor-Gene Q Qiagen. Please contact us regarding suitability with other thermal cyclers

Consumables:

- Precision pipettes capable of delivering volumes of between 1 µl and 1000 µl
- Nuclease-free filtered tips
- 1.5 ml tubes
- 96-well PCR plates, strips or PCR micro-tubes (optical quality compatible with the thermocycler) and appropriate adhesive films or caps

Reagents:

- Distilled or Nuclease-free water (recommended)

Remarks and precautions

The material used contains less than 0.1% hazardous or carcinogenic substances, thus MSDS sheets are not required. However, it is recommended to take appropriate precautions, as with any biochemical product, and to wear appropriate clothing.

Extraction controls

▪ Positive controls

The IDPPR kit contains the following positive controls:

- Target Positive Control (TPC-PPR):
This control consists of inactivated PPRV vaccine strain, diluted in a virus-negative whole blood, calibrated at between 1 and 100 times the MDL.
This control validates the efficiency of the extraction and amplification process.
This control is prepared and extracted in the same way as the samples.

- Endogenous Non-Target Positive Control (NTPCen):
This control is constitutively present in ruminant cells of the sample to be tested. Its function is to validate (1) cell lysis and (2) amplification of a non-target gene. It also confirms the presence of cells, and gives an indication of the quality of the sample.

- **Negative controls**

It is recommended to include the following negative controls in each run:

- Negative extraction control (NEC)

This control should be prepared and extracted in the same way as samples, but does not contain any target DNA for PPRV. The volume occupied by the sample is replaced by a negative matrix or Nuclease-free water.

- Negative control for amplification (NAC)
This control contains 8 µl of reaction mix (ARM-PPR) and 5 µl of Nuclease-free water. It is included in each analysis cycle to control for the presence of any aerosol contaminants.

- **Extraction of viral RNA**

The viral RNA must be extracted from the sample before being amplified by PCR.
For this, IDvet Genetics offers a range of extraction kits that meet the French standard AFNOR NF U47-600 requirements:

Description	*Product name*	*Product code*
Magnetic bead extraction system	ID Gene™ Mag Fast Extraction Kit	MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50/SPIN250

- **Extraction of the control**

The volume of the control to be extracted are described in the table below:
Important:

- The volume indicated are valid regardless of the extraction system.
- The control must be extracted at the same time as the samples.

Control	*Volume*
TPC-PPR	**50 µl**

*Note: If the **NEC** is prepared with a negative sample matrix, refer to the extraction kit protocol for the matrix in question.*

Amplification protocol

- **Preparation of the real-time PCR amplification reaction**

1. Prepare an experimental plan for the analysis of the samples and controls, being sure to distance the positive control (TPC-PPR) from the other samples.
2. Thaw the IDPPR kit, ideally at 5°C (± 3°C) in a refrigerated rack. Thaw at room temperature 21°C (± 5°C) only if the mix is to be used immediately after thawing.
3. Homogenise the contents of the **ARM-PPR** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-PPR** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Add the following to the reaction mix :
   - 5 µl of RNA extracted from each sample to be analysed
   - 5 µl of RNA extracted from the TPC-PPR
   - 5 µl of extracted NEC
   - 5 µl of Nuclease-free water (NAC)
6. Cover the plate or strips with appropriate adhesive film or caps.

- **Programming the amplification phase**

1. Program the thermal cycler detectors to read the following wavelengths for each well:

Target	*Channel capable of reading*	*Quencher*
Sequence specific to PPRV	FAM	non fluorescent *
Sequence specific to ruminant cells NTPCen	HEX/VIC	non fluorescent * (compatible HEX/VIC)

Note: For devices requiring an internal reference for optical calibration, the amplification mix ARM-PPR contains ROX.
**Using a TAMRA™ quencher can improve the data analysis with some instruments.*

2. Choose between the two different amplification programs validated by IDvet Genetics:
- standard program (allows for PCR kits from different suppliers to be used in a single run)

or,

- rapid program

Step		Standard program	Rapid program	Number of cycles
(1)	*Reverse transcription*	10 min at 45°C	10 min at 45°C	1
(2)	*Polymerase activation*	10 min at 95°C	2 min at 95°C	1
(3)	*DNA denaturation/elongation*	15 sec at 95°C 60 sec at 60°C	10 sec at 95°C 30 sec at 60°C	40

Note: The fluorescence is read at the end of the elongation phase at 60°C.

3. Enter one or these programs in the thermal cycler and select a final volume of **13 µl per PCR**. If different volumes are combined in a single run, enter the largest volume on the plate.

4. Place the PCR plate, PCR strips or capillaries in the thermocycler and start the program.

Validation and interpretation of results

Assay validation

The analysis of results is based on the Cq (Quantification cycle) value of each sample that is obtained for each fluorescence detector.
The Cq is also known as the Ct value (Cycle Threshold) or Cp value (Crossing Point).
The test is validated according to criteria outlined in the table below. **Results should not be interpreted if any of these criteria are not met**.

Control	Expected result	Acceptability criteria
TPC-PPR	Detected in FAM and VIC/HEX	Refer to the Cq value indicated in the quality control certificate
NTPCen	Detected in VIC/HEX in each sample	Presence of a characteristic curve
NEC	No detection if water used	Complete absence of a characteristic curve
	Detected in VIC/HEX if virus-negative sample used	Presence of a characteristic curve
NAC	No detection	Complete absence of a characteristic curve

Note: TPC-PPR may be used to monitor variations in analytical sensitivity as it is calibrated at between 1 and 100 times the MDL.

Suggested interpretation of results

For each sample, results may be interpreted according to the following criteria:

Sample	PPRV signal	NTPCen signal	Interpretation
Individual	Detected	Detected or not detected	Animal detected as positive for PPRV
	Not detected	Detected	Animal not detected for PPRV
	Not detected	Not detected	A problem occurred during sample distribution or extraction process / PCR reaction was inhibited

Non-validated samples:

- If the NTPCen is not detected but the sample is detected positive for PPRV, consider the sample as positive.

- If the NTPCen is not detected:

- a problem occurred during sample distribution or during the extraction process. In this case, the sample is to be extracted again

or,

- the PCR reaction was inhibited. In this case, perform a new amplification run following the procedure below.

Procedure to follow if the PCR reaction was inhibited:

1. Dilute the extracted DNA 10 times in Nuclease-free water.
2. Repeat the amplification step on 5 µl of this dilution.
3. If the NTPCen is detected, interpret the sample according to table above.
4. If the NTPCen is not detected, re-extract the sample or consider it uninterpretable.

Documentation and support

For questions or technical support, please contact: **support.genetics@id-vet.com**

For additional information, visit **www.id-vet.com**