Certified management system

ID Gene™ Capripox Virus Triplex

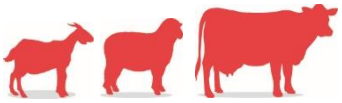
Ref: IDCPV-50 / IDCPV-100

50 / 100 tests

Real-time PCR for the qualitative detection of Capripox viruses including Lumpy Skin Disease, Goatpox and Sheeppox

Suitable samples: Ruminant whole blood, swabs, tissues (skin lesions)

***In-vitro* Use**



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General information

▪ Characteristics

ID Gene™ Capripox Virus Triplex (IDCPV) kit is a real-time PCR kit that amplifies a target sequence in Capripox (CPV) viral genome (including Lumpy Skin disease (LSD), Sheepox and Goatpox viruses).

This kit is a qualitative triplex test. It simultaneously amplifies the target DNA, endogenous and exogenous internal controls.

The kit contains the target positive control (TPC-CPV) and the non-target positive control (NTPC-CPV) which are to be extracted in the same manner as the samples to evaluate extraction efficiency and to detect the presence of PCR inhibitors.

This kit can be used to test ruminant whole blood collected in EDTA, swabs, tissues (skin lesions).

▪ Kit composition and storage conditions

The IDCPV kit contains the reagents shown below:

Reference	*Component*	*Volume*	*Description*
TPC-CPV	Target Positive control	550 µl 1 vial	Inactivated Neethling vaccine diluted in a virus-negative matrix, freeze-dried and calibrated between 10 and 100 times the detection limit of the method (MDL). Freeze-dried pellet to be reconstituted in **550 µl** distilled or Nuclease-free water.
NTPC-CPV	Non-Target Positive Control	2200 µl 1 vial	Non-pathogenic inactivated viral strain. Freeze-dried pellet to be reconstituted in **2200 µl** distilled or Nuclease-free water.
ARM-CPV	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mix containing Taq polymerase and oligonucleotides for detection of CPV and of the endogenous and exogenous non-target positive controls.

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 (> 3 not recommended).

▪ Required equipment, not provided in the kit

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

Amplification Instrument:

- Real-time thermal cycler capable of reading the following wavelengths: 525 nm (FAM), 548 nm (Yakima Yellow, equivalent to VIC) and 650 nm (Cy5).
  Examples of compatible thermal cyclers: CFX96, Chromo4 Biorad, LC480 I, LC480 II, LC96 Roche, 7500 AB 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 (that have an optical quality compatible with the thermal cycler) and appropriate adapted adhesive film or caps

Reagents:

- Distilled or Nuclease-free water (recommended)

Contact genetics@id-vet.com for more information.

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 and amplification controls

▪ Positive controls

The IDCPV kit contains the following positive controls:

- Exogenous Non-Target Positive Control (NTPC-CPV):

The exogenous positive control is a non-pathogenic inactivated DNA virus.
This control evaluates the efficiency of the extraction process and detects the presence of inhibitors in the amplification step.
This control is to be added to every sample as well as to the other controls (NEC) before extraction.

- Target Positive Control (TPC-CPV):

These controls consist of inactivated Neethling vaccine diluted in a virus-negative matrix, calibrated between 10 and 100 times the MDL.
These controls validates the extraction and amplification of the target.
These controls are prepared and extracted in the same way as samples.

- Endogenous Non-Target Positive Control (NTPCen):

This control is constitutively present in the cells of the test sample. 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. The volume occupied by the sample is replaced by a non-reactive matrix or Nuclease-free water.

- Negative control for amplification (NAC)

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

Amplification protocol

- **Extraction of viral DNA**

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

Description	*Product name*	*Product code*
Magnetic bead extraction system	ID Gene™ Mag Universal Extraction Kit	MAG192/MAG384
	ID Gene™ Mag Fast Extraction Kit	MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50/SPIN250

- **Extraction of the controls**

The volumes of the control to extract are described in the table below:

Important:

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

Control	*Volume*
TPC-CPV	**50 µl**
NTPC-CPV	**20 µl** to add to NEC and to each sample

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

- **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-CPV) from the other samples.
2. Thaw the IDCPV 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-CPV** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-CPV** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Add :
   - 5 µl of DNA extracted from each sample to be analyzed
   - 5 µl of DNA extracted from the TPC-CPV
   - 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	*Fluorophore*	*λ (nm)*	*Quencher*
CPV	FAM	495-525	non fluorescent
NTPC-CPV	Cy5	649	non fluorescent
NTPCen	VIC / Yakima Yellow	426-548	non fluorescent (compatible VIC/HEX)

Note: For devices requiring an internal normalization of the signal, the amplification mix ARM-CPV contains ROX.

2. Choose between the two 2 different amplification programs validated by IDvet Genetics:
   - Standard program (allows for PCR kits from different vendors to be used in a single session) or
   - Rapid program

Step	*Standard program*	*Rapid program*	*Number of cycles*
(1) Polymerase activation	10 min at 95°C	2 min at 95°C	1
(2) 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 step 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 thermal cycler 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 by each detector. The Cq is also known as the Ct value (Threshold cycle).

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-CPV	Detected in FAM	Refer to the Cq value given in the quality control certificate
NTPC-CPV	Detected in Cy5 in each sample	+ 3 Cq of control reported to Cq of NEC
NTPCen	Detected in VIC in each sample	Presence of a characteristic curve
NEC	Detected in Cy5 if water used	+ 3 Cq of control with respect to the Cq value indicated in the quality control certificate
	Detected in Cy5 and VIC if virus-negative sample used	Presence of a characteristic curve
NAC	No detection	Complete absence of a characteristic curve

▪ Suggested interpretation of results

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

Sample	*CPV signal*	*NTPCen signal*	*NTPC-CPV signal*	*Interpretation*
Individual	Detected	Detected or Not detected	Detected or Not detected	Animal detected as positive for CPV
	Not detected	Detected	Detected	Animal not detected for CPV
	Not detected	Not detected	Not detected or $Cq_{NTPC\text{-}CPV} > Cq_{NEC} + 3$	A problem occurred during sample distribution or extraction process / PCR reaction was inhibited

Non-validated samples:

- If the NTPC-CPV or NTPCen are not detected but the sample is detected positive for CPV, 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.

- If the NTPC-CPV 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.

- If $Cq_{NTPC\text{-}CPV} > Cq_{NEC} + 3$ and no signal is detected for CPV, the reaction is 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 phase on 5 µl of this dilution.
3. If the NTPC and NTPCen are detected, interpret the sample according to table above.
4. If the NTPC and NTPCen are 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**