


# MONKEYPOX REALTIME PCR KIT

<b>REF</b>	RTPCR018-LPD-R		96
<b>RUO</b>	For research use only		

## INTENDED PURPOSE

Real Time RT-PCR kit to detect nucleic acid from Monkeypox virus (MPXV) in skin lesions. The kit uses two targets, one specific for the Monkeypox virus and generic for *Orthopoxvirus* genus.

## INTRODUCTION

Monkeypox virus is a double-stranded DNA virus, a member of the *Orthopoxvirus* initially identified in monkeys, but it could be mainly found in rodents. In humans, this zoonotic virus, was rarely detected until very recently in countries outside where it is endemic (Central Africa). However, from May 2022, an outbreak in a number of other countries, initially European, and linked to men who have sex with men was detected in other latitudes, reporting for the first-time local transmission without epidemiological links to Africa.

Monkeypox virus belongs to the genus *Orthopoxvirus*, as variola virus (causing smallpox), vaccinia virus or cowpox among others. There are two known clades of MPXV, one endemic in Central Africa (Congo Basin region) and one in Western Africa.

## TEST PRINCIPLE

It is based on amplification and detection of a specific fragment of the Monkeypox virus (MPXV) and a generic fragment for *Orthopoxvirus* genus by real time PCR. One lyophilized master mix (RT-PCR MIX) is provided for detection of viral DNA and the internal control included.

PCR mix targets *F3L* (FAM) as a specific fragment of Monkeypox virus, presents in both clades, and the *E9L* target which is generic of *Orthopoxvirus* (HEX/VIC). Additionally, an amplification control is included associated to the extraction of the sample (human *RNAse P* gene) to check the absence of carry-over of amplification inhibitors and the correct reverse transcription and amplification set-up.

The Monkeypox virus DNA is detected in channels FAM and HEX/VIC, while the internal control is labelled with Cy5.

## KIT FEATURES

VIRCELL RT-PCR MIX and VIRCELL POSITIVE CONTROL are lyophilized. It is necessary to reconstitute them before use (see "Preparatory treatment of the device" section). The rest of the reagents are ready to use. This kit is based on amplification and detection using real time PCR.

## MATERIALS PROVIDED

- [1] VIRCELL MPX RT-PCR MIX LPD: 1 plate with 96 tubes divisible into 12 strips with 8 tubes containing, Taq polymerase, buffer and specific primers/probe for Monkeypox virus and *Orthopoxvirus*. Also, as internal control, primers/probe for human *RNAse P* gene. 1 reaction per tube. Lyophilized.
- [3] VIRCELL MPX POSITIVE CONTROL: 1 vial containing a mixture of lyophilized non-infectious nucleic acids to be used as positive control. Red cap.
- [4] VIRCELL NEGATIVE CONTROL: 200 µl of deionized water to be used as negative control. Green cap.
- [5] VIRCELL PCR MIX RECONSTITUTION SOLUTION: 2 x 1 ml of aqueous solution to reconstitute the PCR mix. Yellow cap.
- [6] VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION: 500 µl of aqueous solution to reconstitute the positive control. Brown cap.
- [7] VIRCELL RT-PCR MIX CAPS: 12 strips of 8 caps RT-PCR compatible.

### Special materials required but not provided:

- Microbiological safety cabinet.
- DNA/RNA extraction kit (see recommendations in "Assay procedure").
- Real Time PCR thermocycler.
- Precision micropipettes.
- Sterile tips with aerosol barrier.
- Microcentrifuge.
- PCR cabinet (recommended).
- Vortexer.

## STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

## IN-USE STABILITY

VIRCELL POSITIVE CONTROL reconstituted: at temperature between -25°C and -15°C and use until expiration date. Avoid multiple freeze-thaw cycles.  
 VIRCELL RT-PCR MIX reconstituted: at temperature between -25°C and -15°C and use until expiration date. Avoid multiple freeze-thaw cycles.  
 Rest of reagents: Refer to package label for expiration date (at 2-8°C).  
 VIRCELL RT-PCR MIX should be used immediately after reconstitution maintaining in a cold rack protected from light.  
 VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

## WARNINGS AND PRECAUTIONS

1. For research use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
11. Keep containers for samples and reagents closed while they are not being handled.
12. Avoid using samples subjected to repeated freeze-thaw cycles.
13. Handle in aseptic conditions to avoid microbial contaminations.
14. Reagents in this kit could include genetic material or substances of animal and/or human origin. Although that material is not infectious, it should be handled as potentially infectious. All material should be handled and disposed as potentially infectious. Observe the local regulations for waste disposal.
15. Dispose of unused reagents and waste in accordance with all applicable regulations.
16. Use kit components only. Do not mix components from different kits or manufacturers. Only VIRCELL NEGATIVE CONTROL, VIRCELL PCR MIX RECONSTITUTION SOLUTION and VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION are compatible with the equivalents in other RTPCR VIRCELL references and lots.
17. Specimens should be handled as in the case of infectious samples using safety laboratory procedures. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
18. Testing of all the samples at the earliest interval following collection will help ensure the most accurate test results. Variation in storage times during specimen shipment has not been assessed.
19. It is recommended to have two different areas to perform the test: Pre-Amplification and Amplification areas.
20. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored to purity.
21. It is recommended to use conventional DNA purification kits.

## CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

The most used samples are those collected from the lesion, such as vesicular swabs collected on a transport medium or dry swab.

Do not delay transport and laboratory investigations. Specimens could be stored at 2-8°C for up to 72 hours after collection, if delay is expected storage between -25 °C and -15 °C is recommended.

## PREPARATORY TREATMENT OF THE DEVICE

All reagents supplied are ready to use, except for the VIRCELL RT-PCR MIX [1] and the VIRCELL POSITIVE CONTROL [3].

[1] VIRCELL RT-PCR MIX. For reconstitution add 15 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per tube.

⚠ The reconstituted VIRCELL RT-PCR MIX must be used right after adding the reconstitution solution and it should be kept in a freeze rack until use protected from light.

[3] VIRCELL POSITIVE CONTROL. Follow the next steps to reconstitute it:

- Centrifuge the corresponding tube for 5 seconds at 5000 g.
- Add 100 µl of VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION [6]
- Mix with vortex for 1-2 seconds.
- Centrifuge the tube for 5 seconds at 5000 g.

After reconstitution, the VIRCELL POSITIVE CONTROL [3] can be frozen at temperature between -25°C and -15°C to be used in subsequent reactions.

## ASSAY PROCEDURE

1. DNA extraction (performed in the Pre-Amplification area):
  - 1.1. It is recommended to use a commercial extraction kit for DNA extraction. In order to use commercial extraction kits, follow the manufacturer instructions. Consult with Customer Service.
2. Amplification using RT-PCR (performed in the Amplification area):
  - 2.1. Preparation of the VIRCELL RT-PCR MIX tubes: Add 15 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per tube. Maintain cold after reconstitution/thawing.
  - 2.2. Addition of the sample: Add 5 µl of each extracted DNA/RNA sample to each tube. Add 5 µl of VIRCELL POSITIVE CONTROL [3] and VIRCELL NEGATIVE CONTROL [4] to the corresponding tubes. The negative control is water.
  - 2.3. Secure caps VIRCELL RT-PCR MIX CAPS [7] on the tubes.
  - 2.4. It is recommended to briefly centrifuge the plate/strips of tubes with the purpose of ensuring vial content is at the bottom of the tube.

CONTROL	MPXV (FAM)	OPXV (HEX/VIC)	IC (Cy5)	Interpretation
VIRCELL MPX POSITIVE CONTROL	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Correct
	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Invalid
VIRCELL NEGATIVE CONTROL	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >38	Correct
	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Invalid

## INTERPRETATION OF RESULTS

The result interpretation is described in the table below:

RESULT	MPXV (FAM)	OPXV (HEX/VIC)	IC (Cy5) <sup>1</sup>	Interpretation
1	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Invalid (sample/kit/setup related)
2	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Monkeypox Negative
3	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or No amplification	Monkeypox Positive
4	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40) or No amplification	Inconclusive
5	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or No amplification	Orthopoxvirus Positive

<sup>1</sup> In case of a high copy number of the target nucleic acid, the amplification of the internal control (IC) in results 3 to 5 may be affected. The late amplification or absence of IC amplification does not change the interpretation of the result.

In case of invalid or inconclusive result, it is recommended to re-extract DNA from original specimen and re-test it. In the case of failure of amplification of internal control, improper extraction of nucleic acids or inhibition of amplification could be assumed. Testing a new sample is recommended.

- 2.5. RT-PCR program: Insert the PCR tubes in the real time thermocycler and run the following program\*:

1 cycle	95 °C	3 minutes
45 cycles	95 °C	15 seconds
	60 °C	45 seconds*

\* Fluorescence data (FAM, Cy5 and HEX/VIC) should be collected.

## INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control testing before releasing, complying with strict specifications.

## VALIDATION PROTOCOL FOR USERS

It is recommended to include one negative control in each run performed. The negative control will monitor reagent or environmental contamination.

The positive control is recommended to be included on each run. The positive control monitors for reagent failures and for correct operation of essential procedure.

The thermocycler software is likely to automatically calculate the baseline fluorescence value (threshold) based on the amplification curve for each target (fluorescence detection). Nevertheless, it is recommended to set the thresholds for the different detection channels individually. In order to set a threshold for each target, it is recommended to use as a reference the amplification curves of the positive and negative controls. The threshold should be fixed at the beginning of the exponential reading of fluorescence and above the background signal.

The controls result interpretation is as follows:

## SYMBOLS USED IN LABELS



For research use only



Use-by (expiry date)



Store at x-y °C



Contains sufficient for <n> test



Batch code



Catalogue number



Consult instructions for use



Reconstitute in <X> µl



Manufacturer

Current version Nr.: L-RTPCR018-LPD-R-EN-01

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Updates: New reference