Advanced Molecular Human Monkeypox (MPXV) PCR Kit

CE IVD

Catalogue Number KD919167- 100

Advanced Molecular Diagnostics Ltd is a diagnostics company specialising in the manufacture and supply of molecular biology instruments, reagents and consumables.

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Kit Contents

Item	**KD919167- 100**
MPXV qPCR Master Mix	2 x 1 ml
MPXV Positive control	1 x 0.1 ml
Nuclease-free water	1 x 1 ml

Intended Use

This product is to be used by personnel specially instructed and trained in the *in-vitro* diagnostics procedures such as correct patient sample handling, use of PPE equipment, use of qPCR machines and interpretation of associated data.
This assay is an *in-vitro* PCR test for the qualitative identification of Human Monkeypox Virus. Samples are to be taken from human samples such as Whole Blood , K2EDTA plasma, Serum and Lesion exudate samples from the skin. It is based on the hydrolysis probe detection method and is a highly sensitive qPCR kit.
For *in-vitro* diagnostic use.

Overview

Monkeypox virus (MPXV) is a zoonotic virus species of the subset genus Orthopoxvirus (OPX), from the Poxviridae family. MPXV falls into the same genus category as Cowpox (CPX), Variola (VARV), and Vaccinia (VACV) viruses. Its name, “monkeypox”, dates back to 1958, when the first cases of the disease were recorded in laboratory monkeys being kept for research in Denmark. MPXV causes the disease in both animals and humans. It can be transmitted through animal-to human-contact e.g., animal bites, contact with animal bodily fluids etc., human-to-human contact e.g., touching infected lesions, inhalation of respiratory droplets, and eating infected meat that has not been thoroughly cooked. The incubation period for MPXV is typically 7−14 days, but can range from 5−21 days. Early onset symptoms of the disease are: swollen lymph nodes, aches and pains, fever and chills, headaches, and extreme fatigue. After 1-3 days, patients develop rashes, which progress to lesions, eventually scabbing over and falling off. The disease usually lasts for around 2-4 weeks.

Principles of the test

The ZenaMax Human Monkeypox Virus (MPXV) Kit is designed for the detection of all relevant genotypes against publicly available sequences of MPXV by real-time Polymerase Chain Reaction (PCR). Amplification of unique conserved target genes of the MPXV genome labelled with fluorescent reporter dyes, is followed by detection by the hydrolysis probe method of qPCR. MPXV presence is indicated by the **FAM** fluorophore.

For the DNA isolation quality control and possible PCR inhibition control there are primers and probe for internal control gene amplification present in the reaction mix. Amplification of internal control gene is indicated in the **HEX** fluorophore fluorescence channel. These primers and probes are incorporated into the ready-to-use PCR master mix.

The point at which the fluorescence becomes detectable above the background, the quantification cycle (Cq), is proportional to the amount of target present in the sample. The lower the Cq, the greater the amount of target present. If the virus in question is not present, the signal will not be produced.

The detection kit utilizes the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity.

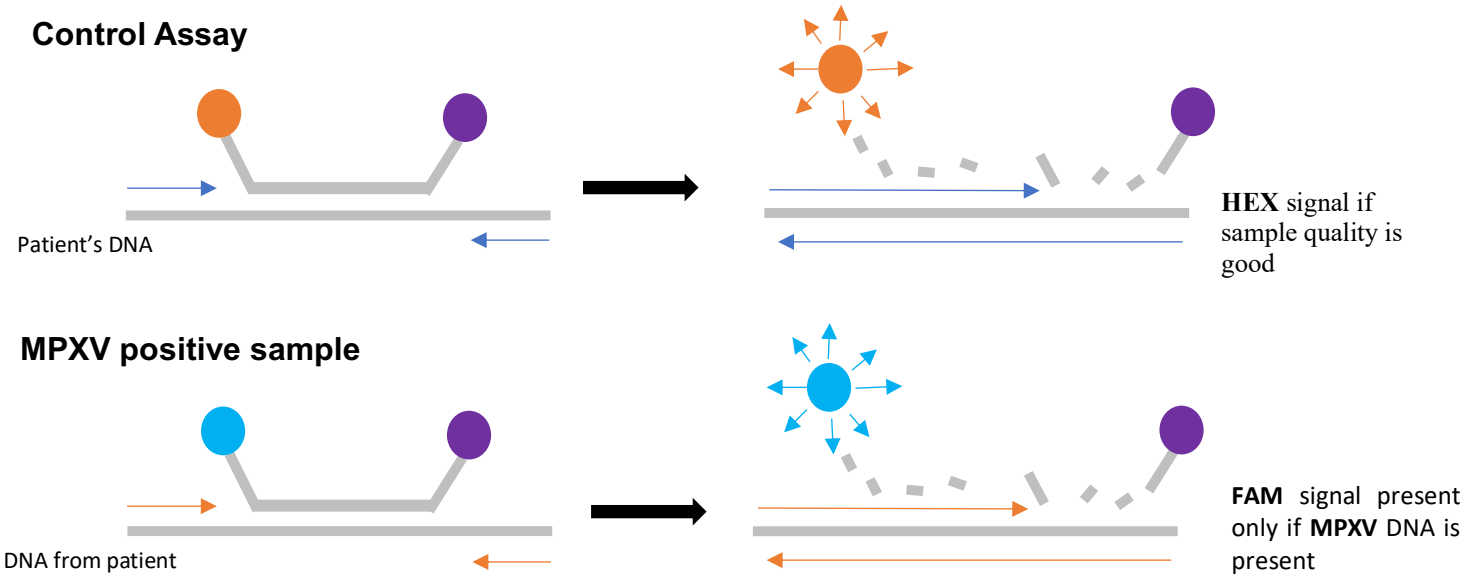

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of viral DNA. The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable.

If a virus **positive** sample is tested, the MPXV Virus assay will produce a FAM fluorescent signal to indicate the viral DNA is present in the patient sample.
Due to assay competition, the HEX signal may be reduced or absent when other signals are strong.
If a virus **negative** sample is tested, only the HEX signal will be detected.

Reagent storage and handling

The kits should be transported and stored at temperatures between -18°C and -10°C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freeze thawing of the kit components may result in lower detection quality. It is recommended that the master mix is aliquoted to avoid this. Avoid exposure to light. Ensure that all reagents are thoroughly thawed, mixed and pulse centrifuged before use.
DNA extraction: For optimal results use a suitable DNA Extraction Kit to elute the DNA from the sample. Other leading kits, or in-house methods are acceptable for use with this diagnostic kit, providing that it has been validated prior to use on patient samples.

PCR Instrument: This kit should be used with qPCR systems which can detect FAM and HEX fluorescent dyes.

Consumables: Use nuclease free PCR consumables appropriate to the qPCR instrument.

Other Laboratory Equipment: Vortex, micro centrifuge, micro pipettes and tips, microfuge tube rack, PCR tube/plate rack.

Warnings and Precautions

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). Discard sample and assay waste according to your local safety regulations. It is essential to precisely follow the instructions in this manual, to ensure accurate results. Please familiarise yourself with this product manual and your qPCR instrument before using the AMD MPXV PCR Detection Kit.

Instrument compatibility

AMD MPXV qPCR detection kit is compatible with the most common Real Time qPCR equipment with the capability of detecting FAM and HEX fluorescent dyes such as Biorad CFX96, Applied Biosystems 7500 Fast, QuantStudio 3,5,7, StepOne Plus, Aglient Mx3000,

3005P, Rotorgene Q, Cepheid Smartcycler, Analytik Jena qTower and Roche Lightcycler 480, 96.

Assay Procedure

Sample Collection

The sample for AMD Human Monkeypox Virus (MPXV) qPCR detection Kit should be collected via human samples such as whole ~~b~~lood , K2EDTA plasma, and lesion exudate samples from the skin. Please ensure that samples are stored correctly and kept away from any contamination.

Sample Storage

As a general rule, multiple freeze-thaws should be avoided. The most practical way to address this concern is by aliquoting samples after collection.

Sample transport

Sample material should be transported in a shatterproof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided. The samples should be transported following the local and national instructions for the transport of pathogen material. The samples should be shipped within 6 hours. It is not recommended to store the samples where they have been collected. It is possible to ship the samples by mail, following the legal instructions for the transport of pathogen material. We recommend the sample transport with a courier.

PCR Set up

Ensure that all reagents and samples are thawed completely, mixed and briefly centrifuged. Keep all reagents and samples on ice during this procedure.
Set up the reactions using the table below.

Product	Volume
DNA MasterMix	20µl
DNA Sample/control	5µl

Add the DNA samples and at least one replicate of the MPXV control to the PCR tubes/plate. As a No Template Control (NTC), add 5µl nuclease free water in place of DNA. Seal the PCR tubes or plate and briefly spin to ensure that the reagents are at the bottom and no air bubbles are present.

Place the plate/tubes in the qPCR thermal cycler and use the following thermal profile:

Thermal Profile:

Stage/Step	Temperature	Time
Stage 1: Step 1	40°C	5 min
Stage 1: Step 2	95°C	5 min
40 Cycles		
Stage 2: Step 1	95°C	10 Sec
Stage 2: Step 2	58°C	30 Sec

When the run has finished, dispose of the PCR reaction tubes/plate in an appropriate manner in accordance with local and national regulations.

Data Analysis

Analyse the data if the software does not do this automatically at the end of the run. Export the data to Excel or a PDF report, depending on the qPCR instrument used, and view the results.

Interpretation of Results

This is a qualitative assay which indicates the presence or absence of Human Monkeypox Virus.

The results should be interpreted as follows:

If there is a signal in the **HEX** channel and no signal in **FAM**, the sample is negative for MPXV.

If there is a signal in the **HEX** and **FAM** channels, the sample is positive for MPXV.

The internal control assay signal in the **HEX** channel should be present, but may be absent or have a high Cq value (low signal) when the diagnostic assay (**FAM**) signal is strong. This is an inconclusive result. If there is no signal in either channel, the result is also inconclusive. Inconclusive or ambiguous sample results should be repeated.

HEX (Internal control)	**FAM** (MPXV)	**Interpretation**
+	-	Sample Negative for MPXV
+	+	Sample Positive for MPXV
-	+	Sample Positive for-MPXV
-	-	Result inconclusive

Performance Characteristics

Quality: All AMD kits are manufactured under high quality standard methods and unique precision, comparable with other leading commercial Human MPXV PCR Detection Kits.
Sensitivity: The AMD MPXV PCR detection kit is highly sensitive, able to detect a minimum 10 copies/rxn "rxn volume 25µl" under our validation methods and devices.

Specificity: Advanced Molecular Diagnostics Human MPXV kit is very specific up to 100% for Human Monkeypox Virus DNA under our validation methods and devices.

References

Durski, K. N. et al. Emergence of monkeypox —West and Central Africa, 1970 – 2017. MMWR Morb. Mortal. Wkly. Rep. 67 ,306–310 (2018).

Oliveira, G., Rodrigues, R., Lima, M., Drumond, B. & Abrahão, J. Poxvirus host range genes and virus-host spectrum: a critical review. Viruses 9, 331 (2017).

Oldal, M. et al. Serologic survey of orthopoxvirus infection among rodents in Hungary. Vector-Borne Zoonotic Dis. 15, 317–322 (2015).

Product Limitations

This kit is for in vitro diagnostic procedures and should only be used by specifically trained laboratory personnel. The expiry date of all components must be checked before use and disposed of if expired.

Occasionally mutations may arise in the region of the genome targeted by the primers and probes of this assay, leading to under-quantification or failure to detect the presence of the virus in these cases. Assay design and efficacy is reviewed periodically.

Additional Information

AMD produces real-time PCR kits with a wide range of applications for researchers from gene expression analysis, cDNA and population genotyping studies to the multiplex detection of several disease targets real-time PCR with excellent sensitivity and specificity.

Contact

Any queries, comments or complaints please refer to our website at:

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