MD0495_IFU_en_V2401

Monkeypox virus Real-time PCR Kit

SKU	Presentation
MD04951	96 reactions

Description

Monkeypox is a rare disease caused by infection with the monkeypox virus. This virus is an enveloped double-stranded DNA virus, a member of the *Orthopoxvirus* genus in the family *Poxviridae*. The *Orthopoxvirus* genus also includes variola virus (that causes smallpox), vaccinia virus (used in the smallpox vaccine) and cowpox virus. Poxviruses can cause disease in humans and many other animals. Monkeypox is transmitted to humans through close contact with infected person or animal (viral zoonosis), or with material contaminated with the virus. Infection typically results in the formation of lesions, skin nodules or disseminated rash. Monkeypox virus Real-time PCR Kit is designed for the *in vitro* detection of monkeypox virus genomes. The primers and probe sequences have very high (100%) homology with a broad range of Monkeypox virus genomes based on a comprehensive bioinformatic analysis with all reference data within the NCBI database at the time of the design. Other closely related species are not detected. This kit was meticulously designed and validated to meet the rigorous criteria of a quantitative assay. However, it is important to note that the provided Positive Control is not intended for quantification purposes. We recommend checking NZYtech website for the availability of a suitable Quantitative Standard for an accurate quantification. In alternative, commercially genomic DNA standards can also be used. If you require further information or have a specific question about the detection profile of this kit, please send an e-mail to info@nzytech.com and our scientific team will answer your question. This kit is designed to be used by trained users in a suitable molecular biology laboratory environment.

Shipping & Storage Conditions

This product is shipped refrigerated. All components should be immediately stored at -85°C to -15°C upon arrival. When in use, the kit components should be returned to the freezer promptly after use to minimise the time at room temperature. Also proceed with the following recommendations:

- Minimise the number of freeze-thaw cycles by storing kit components in working aliquots. If appropriate, kit components may be aliquoted into smaller volumes after thawing.
- The PPMix must be stored protected from light. Particularly, do not expose the NZYSupreme qPCR Probe Master Mix (2x) to direct sunlight after combining it with the PPMix.
- If the package that protects the kit arrived damaged, please contact NZYtech.
- Beware of the expiry date indicated on the packaging. NZYtech does not recommend using the kit after the expiry date. On this date, the kit must be discarded.

Components

The kit provides a comprehensive set of reagents sufficient to perform 96 *in vitro* Real-time PCR reactions.

COMPONENT	TUBES	VOLUME
Monkeypox MMix	1	1050 µL
Monkeypox PPMix *	1	205 µL
NTC	1	105 µL
Monkeypox POS ¥	1	105 µL

* Monkeypox virus (B7R & CrmB genes) / ACTB PPMix (FAM™, HEX™ and Cy5™ labelled, respectively).
¥ Monkeypox virus (B7R & CmrB genes / ACTB Positive Control

Reagents, Materials and Equipment Required but Not Provided

- Real-time PCR Instrument that detects FAM™, HEX™/JOE™/VIC™ and Cy5™ fluorescent dyes (emission wavelengths of 520, 556/555/554 nm and 682 nm, respectively).
- DNA extraction kit: we recommend using NZYtech's DNA extraction kits which are constantly fine-tuned to optimize Molecular Diagnostic applications.
- Quantitative Standard for quantification of Monkeypox virus.
- RNase/DNase-free qPCR plasticware: PCR tubes, strips, caps, 96-well plates, and adhesive films (also available at NZYtech).
- Pipettes and filter tips (RNase/DNase-free).
- Disposable gloves.
- Vortex and centrifuge.

Sample Material

Monkeypox virus Real-time PCR Kit is used for detection of monkeypox virus usually identified in serum or lesion exudate samples by using real-time PCR systems. All DNA samples that are suitable for qPCR amplification may be used with this kit. However, different factors such as protocol for sample collection from biologic material, transport, storage, and processing time are critical to achieve optimal results. Please ensure DNA samples are suitable in terms of purity, concentration, and nucleic acid integrity.

The Dynamic Range of the Test

Under optimal PCR conditions this NZYtech Molecular Diagnostic Real-time PCR Kit displays high priming efficiencies (>95%) and can detect at least 10 copies of the target template per reaction using different samples.

Rational for the test

Real-time PCR

NZYtech Monkeypox virus Real-time PCR Kit includes all reagents required to identify the presence of monkeypox virus DNA in a variety of relevant samples. DNA isolated and purified from biological material is amplified in a single reaction using two highly specific primers/probe sets exploiting the so-called TaqMan® principle. During this process, the probes specifically anneal to two regions of the monkeypox virus genome, namely B7R and CrmB genes, in case the sample was extracted from an infected patient. An additional primers/probe set acts as an endogenous internal control to detect nucleic acids of the human β-actin (ACTB) assessing sample quality. In addition, this internal control demonstrates that no reaction inhibition has occurred by PCR inhibitors potentially present in the clinical/environmental samples. To allow identifying the amplification of the three specific targets in a single reaction, monkeypox virus (B7R and CrmB genes) and human β-actin specific probes are differently labelled, with FAM™, HEX™ and Cy5™ reporter dyes, respectively. Note that this panel contains a duplex assay in two distinct optical channels – FAM and HEX/HOE/VIC - to report performance of the two PCR assays for monkeypox virus detection. In addition, they are provided in optimized concentrations to make sure amplification of human DNA, even when present at very high concentrations, does not limit the efficiency of the monkeypox virus primers/probe sets.

Negative Control

To validate any positive findings, a negative control reaction should be included every time the kit is used. To perform this, the NTC should be used instead of DNA template. A negative result for all channels indicates that the reagents have not become contaminated while setting up the run.

Positive control (POS)

The kit includes a Positive control template that allows controlling the performance of qPCR reactions. This control corresponds to a synthetic nucleic acid molecule carrying sequences that are homologous to monkeypox virus (B7R & CrmB genes)/ACTB targeted sequences included in this detection assay. The Positive control should be used directly with the primers/probe mix each time an array of samples is tested. A positive result indicates that the primers and probe set for detecting the three target genes worked properly in the included master mix in that experimental scenario. If a negative result is obtained, the test results are invalid and must be repeated. Care should be taken to ensure that the Monkeypox POS does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a post-PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the Positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the Positive control into the positive control well. If such sealing is not possible, we suggest pipetting the PC into a well located the furthest possible from the negative control and biological sample wells. Please note that the PC in the kit is a representative sequence associated with the designed target region and does not contain the organism's entire genome.

Standard Protocol

Nucleic Acids Extraction

This kit must be used with DNA isolated usually from serum or lesion exudate samples. The DNA extraction protocol should be performed according to the manufacturer's instructions.

Procedure

1. qPCR reaction mixture

Beware that all pipetting steps and experimental plate set-up should be performed on ice. After the plate is poured proceed immediately to the qPCR protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artefacts that reduce the sensitivity of detection. Before the experiment, start to gently mix the reaction tubes provided, centrifuge for 5 seconds to collect contents at the bottom of the tube and place tubes on ice. **If possible, pipet positive control last to avoid contamination events.**

Prepare the qPCR reaction mixture according to the table below that specifies the volumes for 1 and *n* reactions (*n*, number of reactions). NZYtech highly recommends performing qPCR analyses in duplicates, which increases the probability of detection of the pathogen and facilitates the interpretation of results.

COMPONENT	1 REACTION VOLUME (µL)	*n* REACTIONS * VOLUME (µL)
Monkeypox MMix	10	*n* x 10
Monkeypox PPMix	2	*n* x 2
Final Volume	**12**	*n* x 12

* Include sufficient reactions for the negative and positive(s) controls. For negative control use NTC.

2. Reaction setup

2.1. Pipette 12 µL of each qPCR mix into individual wells according to your real-time PCR experimental plate setup.

2.2. For the negative control reaction (**mandatory**), add 8 µL of NTC instead of the DNA template into the negative control well. The final volume in each well is 20 µL.
Note 1: *Negative controls should be prepared and properly sealed before the addition of the biological samples and positive controls. If this is not possible, avoid pipetting the negative control in adjacent wells to the positive control and biological samples.*

2.3. For the biological sample(s) reaction(s), pipette 8 µL of each extracted DNA sample into the corresponding wells, according to your experimental plate setup. The final volume in each well should be 20 µL.
Note 2: *Seal all biological samples and negative controls before pipetting the PC into the positive control well. If not possible, avoid pipetting the positive and negative controls and the biological samples in adjacent wells.*

2.4. For the positive control reaction (**mandatory**), add 8 µL of Monkeypox POS into the corresponding well. The final volume in each well should be 20 µL.

Suggested thermal cycling conditions

NZYSupreme qPCR Probe Master Mix (2x) is an optimized and highly efficient reaction mixture developed for real-time PCR. The table below displays a standard protocol optimized on several platforms. However, these conditions may be adapted to suit different machine-specific protocols.

CYCLES	TEMPERATURE	TIME	NOTES
1	95 °C	2 min	Polymerase activation
40	95 °C	5 s	Denaturation
	60 °C	30 s	Annealing/Extension*

* Fluorogenic data should be collected during this step through the FAM, HEX/JOE/VIC and Cy5 channels.

Quality Control

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through real-time qPCR.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the kit component in test for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the kit component in test for 1 h at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids. To test DNases or RNases contamination of the nucleic acid controls, dilutions of the controls are incubated for 14-16 h at 37 °C and at -20 °C. After incubation, a qPCR/RT-qPCR reaction is performed comparing Ct values of the samples incubated at 37 °C and at -20 °C. There must be a deviation of less than 2 Cts between the two samples.

Functional assay

The qPCR/RT-qPCR reactions must ensure the consistent amplification of target DNA/RNA and internal extraction control across serial dilutions, meeting specified acceptance criteria for assay performance.

Data analysis

Before analysing sample results, we recommend verifying if the real-time PCR test is valid. Thus, for each plate, please confirm if the results for Positive and Negative controls performed as expected, according to the following criteria:

- **Positive control (POS)**: the amplification curves of FAM (for B7R gene), HEX/JOE/VIC (for CrmB gene) and Cy5 (for ACTB) are positive. The positive control is expected to amplify with a Cq <30, in the FAM, HEX/JOE/VIC and Cy5 channels. *Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised. Repeat the test.*
- **Negative control (No Template Control, NTC reaction)**: no amplification is detected. If the negative control has one, two or three amplification curves (FAM, HEX/JOE/VIC and/or Cy5) with a sigmoidal shape, sample contamination may have occurred. *Repeat the test following good qPCR practices.*

After verification of the validity of the test, use the following table for the interpretation of principal results (evaluate the overall shape of the amplification curves; only sigmoidal amplification curves are indicative of true amplification).

Sample Target Cq < 36 (B7R gene – FAM)	**Sample Target Cq < 36** (CrmB gene – HEX)	**Sample Target Cq < 40** (ACTB – Cy5)	**Negative Control Cq > 40**	**Positive Control Cq < 30**	**Result**
+	+	+	-	+	**POSITIVE result for Monkeypox virus**
+	-	+	-	+	**POSITIVE result for Monkeypox virus**
-	+	+	-	+	**INCONCLUSIVE result → Monkeypox virus/other *Orthopoxvirus* detected only for one target**
-	-	+	-	+	**NEGATIVE result**