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TaqPath™ Monkeypox/Orthopox Virus DNA Kit

INSTRUCTIONS FOR USE

Multiplex real-time PCR test intended for the qualitative detection of DNA from Monkeypox/Orthopox virus and RNase P internal control in a single reaction well

Catalog Number A56978

Publication Number MAN0028390

Revision A.0

IVD

For In Vitro Diagnostic Use. For Emergency Use Authorization Only | Rx Only

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: A.0 MAN0028390 (English)

Revision	Date	Description
A.0	14 December 2022	New instructions for use for the TaqPath™ Monkeypox/Orthopox Virus DNA Kit.

The customer is responsible for validation of assays and compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

The information in this guide is subject to change without notice.

Contents

1 Product information

Intended Use

The Applied Biosystems™ TaqPath™ Monkeypox/Orthopox Virus DNA Kit is a multiplexed polymerase chain reaction (PCR) test intended for the qualitative detection of DNA from monkeypox virus (clade I/II) and non-variola *Orthopoxvirus* in human lesion swab specimens (i.e., swabs of acute pustular and vesicular rash) from individuals suspected of mpox by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of monkeypox virus (clade I/II) and non-variola *Orthopoxvirus* DNA which is generally detectable in human pustular or vesicular lesion specimens during the acute phase of infection. Positive results are indicative of the presence of monkeypox virus (clade I/II) DNA and/or other non-vriola *Orthopoxvirus* DNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results obtained with this device do not preclude monkeypox virus (clade I/II) and/or non-variola *Orthopoxvirus* infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Laboratories within the United States and its territories are required to report test results to the appropriate public health authorities.

The Applied Biosystems™ TaqPath™ Monkeypox/Orthopox Virus DNA Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The Applied Biosystems™ TaqPath™ Monkeypox/Orthopox Virus DNA Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product description

The TaqPath™ Monkeypox/Orthopox Virus DNA Kit is a multiplexed real-time PCR testing solution that can detect nucleic acid (DNA) from monkeypox virus (MPXV), non-variola *Orthopoxvirus* (OPXV) targets, and human RNase P in a single reaction well.

Each kit includes the following components:

- TaqPath™ Monkeypox/Orthopox Virus Multiplex Assay—Multiplexed real-time PCR assay containing primers/probes specific to monkeypox virus, non-variola *Orthopoxvirus* targets, and human RNase P.
- TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control—Positive DNA control that contains templates specific to monkeypox virus, non-variola *Orthopoxvirus* targets, and human RNase P regions targeted by the assay.

TaqPath™ BactoPure™ Microbial Detection Master Mix (No ROX™) and TE Buffer must be ordered separately (see “Required materials not supplied” on page 8).

The assay contains primers and probe sets specific to the following targets:

- Monkeypox virus unannotated region
- Non-variola *Orthopoxvirus* DNA polymerase gene
- RNase P (human sample collection control)

Table 1 Dyes, quenchers, and targets

Target	Dye	Quencher
Monkeypox virus	FAM™ dye	None[1]
Non-variola *Orthopoxvirus*	VIC™ dye	
RNase P	JUN™ dye	

[1] The TaqPath™ Monkeypox/Orthopox Virus DNA Kit probes contain QSY™ and MGB quenchers, which do not fluoresce. Select **None** for **Quencher** in the instrument set up procedure (see page 27, page 30, or page 34).

Results are analyzed using the following software:

- Applied Biosystems™ Pathogen Interpretive Software v1.1
- SAE Administrator Console Dx v1.0 or SAE Administrator Console Dx v1.2 (for security and audit functions)
- Pathogen Interpretive Software 1.0.0 DAT (SAE Profile)
- The appropriate assay panel for your instrument:
  - MPXOPX-EUA_QS5-9601_1.0.0.zip
  - MPXOPX-EUA_QS5Dx-9602_1.0.0.zip
  - MPXOPX-EUA_QS7F-384_1.0.0.zip

For more information, see Chapter 7, “Analysis and results”.

Contents and storage

Table 2 TaqPath™ Monkeypox/Orthopox Virus DNA Kit, 200 reactions (Cat. No. A56978)

Component	Quantity	Amount per tube or bottle	Storage
TaqPath™ Monkeypox/Orthopox Virus Multiplex Assay	1 tube	250 µL	–30°C to –10°C[1]
TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control	1 tube	25 µL	–30°C to –10°C[1]

[1] Do not freeze-thaw more than 5 times.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Real-time PCR instrument (for software compatibility for each instrument, see “Software and instrument compatibility” on page 37)	
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, 96-well, 0.1-mL block	A28573 (with desktop computer)
	A28568 (with laptop computer)
	A28138 (instrument only)
Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR System, 96-well, 0.2-mL block	A47326 (with laptop computer)
	A47327 (with tower computer)
Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System, 384-well	4485695 (with laptop computer)
	4485701 (with desktop computer)
Equipment	
Laboratory freezers, –30°C to –10°C	MLS
BSL-2 biological safety cabinet	MLS
Centrifuge, with a rotor that accommodates standard and deepwell microplates	MLS
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS

(continued)

Item	Source
Single and multichannel adjustable pipettors (1.0 µL to 1.0 mL)	MLS
Cold block (96-well or 384-well) or ice	MLS
Nucleic acid extraction system and materials	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
KingFisher™ Flex 96 Deep-Well Heating Block	24075430
KingFisher™ 96 Deep-Well Plate	95040450, A48305, 95040455
KingFisher™ 96 tip comb for deep-well magnets	A48438, 97002534
96-well plate for the tip comb, one of the following:	
KingFisher™ 96 KF microplate	97002540
Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom	167008
Nunc™ MicroWell™ 96-Well Microplate, barcoded	269787
Abgene™ 96-Well Polypropylene Storage Microplate	AB0796
Abgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate	AB1127
KingFisher™ 96 Deep-Well Plate	95040450, A48305, 95040455
Kits and reagents	
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	A48383
TaqPath™ BactoPure™ Microbial Detection Master Mix (No ROX™)	A52703, A52704, A52705
TE Buffer	12090015
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade[1], or equivalent	BP2818100, BP2818500, BP28184
Nuclease-Free Water (not DEPC-Treated)	MLS
Plates—QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block)	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906, 4366932
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL (without barcode)	4346907
Plates—QuantStudio™ 5 Dx Real-Time PCR Instrument (96-well, 0.2-mL block)	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL	4306737, 4326659
MicroAmp™ Optical 96-Well GPLE Reaction Plates with Barcode, 0.2 mL	4481192
MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL	N8010560, 4316813

(continued)

Item	Source
MicroAmp™ Optical 96-Well GPLE Reaction Plates, 0.2 mL	4481191
MicroAmp™ Optical Film Compression Pad	4312639
Plates—QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)	
MicroAmp™ Optical 384-Well Reaction Plate with Barcode	4309849, 4326270, 4343814
MicroAmp™ Optical 384-Well Reaction Plate	4343370
JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, 384-well	A24733
Tubes and other consumables	
Sterile aerosol barrier (filtered) pipette tips	**thermofisher.com/pipettetips**
Nonstick, DNase and RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	AM12450, AM12475, or equivalent
DNase and RNase-free tubes for mixing reagents (capable of mixing 5 mL)	**thermofisher.com**
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971, 4360954
MicroAmp™ Adhesive Film Applicator	4333183

[1] Available at **fisherscientific.com**.

General laboratory recommendations

- Follow standard operating procedures in your laboratory to prevent contamination, such as the following:
  - Frequent glove changes
  - Frequent decontamination of surfaces, equipment, and pipettes with fresh 10% bleach or decontamination solution, followed by 70% ethanol
  - Use of ultraviolet light during biosafety cabinet decontamination (when available)
- To prevent degradation, keep eluted sample DNA, master mixes, assays, and controls on ice or in cold blocks while in use.
- Limit freeze-thaw cycles.
- Aliquot reagents to prevent stock contamination and reduce the number of freeze-thaw cycles.
- After each run, review the amplification curves for signs of inadequate vortexing or centrifugation.

Assay limitations

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- Performance of the assay has been evaluated using contrived clinical lesion swab specimens. Clinical performance with natural clinical lesion swab specimens has not been established.
- The kit performance was established using lesion swab specimens collected in universal transport media (UTM) and viral transport media (VTM) only. Assay performance has not been evaluated for use with other collection media and/or specimen types. Use of other collection media and/or specimen types may lead to a false positive, false negative, or invalid result.
- While monkeypox virus clade II is the only member of the *Orthopoxvirus* genus known to be circulating among humans in the US at this time, a positive result most likely represents the presence of monkeypox virus clade II. There is a small possibility that a positive result could represent the presence of monkeypox virus clade I. If clinical concern for a clade I infection exists, healthcare providers should contact the CDC and their local public health authorities for guidance.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Chemical inactivation and other known clinically relevant interfering substances and techniques can lead to inappropriate primary samples and interfere with test results.
- The workflow must be performed according to the specified methods described in the instructions for use.
- The quality of the biological samples is essential for the quality of the results generated with this kit.
- This kit uses purified DNA as a sample for the analysis. The quality of the DNA recovered from biological samples is essential for the quality of the results generated with this kit.
- Performance was established using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383). Other purification methods may lead to deviations in the expected result.
- Assay performance was established with a maximum of 5 freeze-thaw cycles. Additional freeze-thaw cycles may result in deviations in the expected results.
- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the monkeypox virus and/or non-variola *Orthopoxvirus* DNA during shipping/storage
  - Using unauthorized extraction methods or assay reagents
  - The presence of PCR inhibitors
  - Mutation in the monkeypox virus and/or non-variola *Orthopoxvirus*
  - Failure to follow instructions for use
  - Absence of specimen

- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - DNA contamination during product handling
- Negative results do not preclude infection with monkeypox virus and/or non-variola *Orthopoxvirus* and should not be the sole basis of a patient management decision. Collection of multiple specimens (and specimens collected at different time points) from the same patient may be necessary to detect the virus.
- Detection of monkeypox virus DNA is dependent on the number of copies present in the specimen. Detection of MPXV DNA may be affected by sample collection methods (e.g., if a specimen is improperly collected, transported, or handled), patient factors (e.g., presence, type, and duration of symptoms), stage of infection (e.g., if collected too early or too late in the course of illness), and/or presence of interfering substances.
- Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs/symptoms and epidemiological risk factors.
- As with any molecular test, mutations within the target regions of TaqPath™ Monkeypox/Orthopox Virus DNA Kit could affect primer and/or probe binding, resulting in failure to detect the presence of virus.
- The clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of monkeypox virus and their prevalence, which change over time.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The TaqPath™ Monkeypox/Orthopox Virus DNA Kit cannot rule out diseases caused by other bacterial or viral pathogens.
- Interfering substances studies have not been performed for this assay. The assay employs conventional well-established nucleic acid extraction methods used for other similar assays. Interference from common endogenous substances is not anticipated.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of monkeypox virus (clade I/II) and/or non-variola *Orthopoxvirus*. All MPXV and OPXV-negative specimens must have a positive RNase P result to be identified as valid negatives.

Warnings and precautions

The TaqPath™ Monkeypox/Orthopox Virus DNA Kit workflow should be performed by qualified and trained staff to avoid the risk of erroneous results. Use separate areas for the preparation of patient samples and controls to prevent false positive results. Samples and reagents must be handled in a biological safety cabinet.

- Samples and controls should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Do not use the kits after the indicated expiry date.
- Dispose of waste in compliance with local, state, and federal regulations.
- Safety Data Sheets are available upon request.
- Positive results are indicative of the presence of monkeypox virus and/or non-variola *Orthopoxvirus* DNA.
- Reagents must be stored and handled as specified in “Contents and storage” on page 8.
- Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act).
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- For Use under Emergency Use Authorization Only.
- For prescription use only.
- For *in vitro* diagnostic use.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories[1].
- This product has been authorized only for the detection of DNA from monkeypox virus or other non-variola orthopoxviruses, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of monkeypox virus, including *in vitro* diagnostics that detect and/or diagnose infection with non-variola *Orthopoxvirus* under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. §360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Laboratories are required to report test results to the appropriate public health authorities.

[1] For ease of reference, this document will refer to "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

Samples and controls

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

- Patient lesion swabs must be collected, transported, and stored in non-inactivating viral transport medium (VTM) according to CDC guidelines. See the CDC website: **https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html**.
- Patient lesion swabs can be collected, transported, and stored in non-inactivating universal transport medium (UTM) under refrigerated conditions (2–8°C) up to 7 days.
- Specimens must be packaged, shipped, and transported according to the current edition of the *International Air Transport Association (IATA) Dangerous Goods Regulations* (**iata.org/en/programs/cargo/dgr**).

Positive and negative test controls must be included and evaluated prior to interpreting patient test results.

Include the following controls:

Control	Used to monitor	Assays
Positive control (TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control)	PCR reaction setup and reagent integrity	Monkeypox, non-variola *Orthopoxvirus*, and RNase P
Negative control	Cross-contamination during DNA extraction and reaction setup	Monkeypox, non-variola *Orthopoxvirus*, and RNase P

Sample stability

Process lesion swab samples immediately or shortly after collection. Samples collected in VTM are stable at 2–8°C for up to 7 days and at -20°C or lower for up to 30 days. Samples collected in UTM are stable at 2–8°C for up to 7 days (see “Specimen stability” on page 51).

In-use reagent stability

Reagent	Stability information
TaqPath™ Monkeypox/Orthopox Virus Multiplex Assay	Once thawed, the TaqPath™ Monkeypox/Orthopox Virus Multiplex Assay is stable for up to 24 hours at 2°C–8°C. Do not exceed 5 freeze/thaw cycles.
TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control	Do not exceed 5 freeze/thaw cycles.
Diluted TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control	Once thawed and diluted, the TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control is stable for up to 24 hours at 2°C–8°C.

Workflow

TaqPath™ Monkeypox/Orthopox Virus DNA Kit workflow

Extract DNA from patient samples (see Chapter 2, Extract DNA)

Perform automated DNA extraction using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with 400-µL sample input volume.

Perform real-time PCR

1. Prepare real-time PCR reactions (see page 21)
2. Perform real-time PCR using one of the following real-time PCR instruments:
   - QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.1-mL block (see page 26)
   - QuantStudio™ 5 Dx Real-Time PCR Instrument, 96-well, 0.2–mL block (see page 29)
   - QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block (see page 33)

Analyze data and interpret the results using the Pathogen Interpretive Software (see Chapter 7, Analysis and results)

1. Install the appropriate assay panel for your instrument (see page 39).
2. Analyze the data (see page 40).
3. Review run control results and interpret patient samples (see page 40).

The workflow begins with DNA extraction from lesion swab specimens that were collected in VTM or UTM according to appropriate laboratory procedures.

DNA is isolated and purified from the specimens using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit. DNA isolation is performed via an automated process using the KingFisher™ Flex Purification System (KingFisher™). For more information about using the kit, see "Related documentation" on page 61.

The DNA is amplified using the TaqPath™ Monkeypox/Orthopox Virus DNA Kit and one of the following real-time PCR instruments:

- QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.1-mL block
- QuantStudio™ 5 Dx Real-Time PCR Instrument, 96-well, 0.2-mL block
- QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block

In the process, the probes anneal to specific target sequences located between unique forward and reverse primers for the following targets:

- Monkeypox virus (unannotated region)
- Non-variola *Orthopoxvirus* (DNA polymerase gene)
- RNase P (human sample collection control)

During the extension phase of the PCR cycle, the 5′ exonuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the real-time PCR instrument.

The data are analyzed, then interpreted by the Pathogen Interpretive Software and the appropriate assay panel for your instrument.

2 Extract DNA

IMPORTANT! The DNA extraction protocols in this chapter are compatible with lesion swab specimens collected in VTM and UTM.

Automated DNA extraction is performed using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head, KingFisher™ Flex 96 Deep-Well Heating Block, and the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with a sample input volume of 400 µL.

Before you begin

Note: During the wash steps, the Wash Solution from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit may develop inert white or brown particulates that float in solution. This is not a cause for concern and does not negatively affect performance.

- Determine the number of required reactions based on the number of patient samples to be processed, plus one negative control per plate.
- Prepare fresh 80% ethanol in accordance with standard laboratory procedures using Ethanol, Absolute, Molecular Biology Grade and nuclease-free water for the required number of reactions, sufficient for 1 mL per reaction, plus 10% overage. See “Required materials not supplied” on page 8.
- Mark the negative control well on the plate.
- Label the short side of each KingFisher™ 96 Deep-Well Plate (4):

Label	Number of plates
Sample Plate	1
Wash 1 Plate	1
Wash 2 Plate	1
Elution Plate	1

- Label the short side of the KingFisher™ 96 KF microplate (1):

Label	Number of plates
Tip Comb Plate	1

Note: The following items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:

- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- Abgene™ 96-Well Polypropylene Storage Microplate
- Abgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate
- KingFisher™ 96 Deep-Well Plate

Set up the instrument

1. Ensure that the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head is set up with the KingFisher™ Flex 96 Deep-Well Heating Block.

   IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the **MVP_2Wash_400_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at **www.thermofisher.com** and loaded onto the instrument.

Prepare the processing plates

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as MicroAmp™ Clear Adhesive Film), then store at room temperature for up to 1 hour while you set up the sample plate.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	2	KingFisher™ 96 Deep-Well Plate	Wash Solution	1,000 µL
Wash 2 Plate	3		80% Ethanol	1,000 µL
Elution Plate	4		Elution Solution	50 µL
Tip Comb Plate	5	Place a KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 KF microplate		

Prepare the Binding Bead Mix

The following procedure uses components from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

WARNING! Do not use bleach or bleached pipette tips with the Binding Bead Mix. The Binding Solution from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit contains guanidium thiocyanate, which produces cyanide gas when combined with bleach.

Prepare fresh Binding Bead Mix on each day of use.

1. Vortex the Binding Beads from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit to ensure that the bead mixture is homogeneous.
2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well[1]
Binding Solution	530 µL
Binding Beads	20 µL
Total volume per well	550 µL

[1] Include 10% overage when making the Binding Bead Mix for use with multiple reactions.

3. Mix well by inversion, then store at room temperature.

Prepare sample plate

WARNING! Do not use bleach or bleached pipette tips with the Binding Bead Mix. The Binding Solution from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit contains guanidium thiocyanate, which produces cyanide gas when combined with bleach.

1. Invert the prepared Binding Bead Mix 5 times gently to mix, then add 550 µL to each sample well and the negative control well in the sample plate.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add Binding Bead Mix to sample wells, as the high viscosity will cause variations in the volumes added.

2. Add 400 µL of sample to each sample well.

   Note: Change tips between samples to reduce cross-contamination.

3. Add 400 µL of nuclease-free water to the negative control well.
4. Add 10 µL of Proteinase K to each sample-containing well in the KingFisher™ 96 Deep-Well Plate labeled "Sample Plate", including the negative control well.

Process the samples

1. Select the **MVP_2Wash_400_Flex** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.
2. Start the run, then load the prepared plates into position when prompted by the instrument.
3. After the run is complete (~25 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately.

   The samples are eluted in 50 µL of Elution Solution (see “Prepare the processing plates” on page 18).
4. Place the Elution Plate on ice for immediate use in real-time PCR.

Note:

- Significant bead carry over may adversely impact PCR performance. If bead carry over is observed, re-extract a new aliquot of the sample.
- To ensure reliable performance of the KingFisher™ Flex Magnetic Particle Processor, perform preventive maintenance as instructed by the manufacturer.

3 Prepare real-time PCR reactions

Guidelines for real-time PCR

IMPORTANT!

- Prepare and keep the run plate on ice (or cold block) until it is loaded into the real-time PCR instrument.
- Run the plate immediately after preparation. Failure to do so could result in degraded DNA samples.
- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and DNA samples, and always use aerosol barrier pipette tips.
- Maintain a DNase-free environment. Periodically decontaminate surfaces.
- Protect assays from light.
- Keep DNA samples and components on ice during use.
- Add samples (templates) to reactions in an area designated for handling templates.
- For each real-time PCR plate, include the following controls:
  - One positive control
  - One negative control from each extraction run.

    For example, if DNA samples from 4 extraction runs are combined on one 384-well real-time PCR plate, then 4 negative control wells must be run on that 384-well real-time PCR plate.

Prepare the real-time PCR reactions (96-well reaction plate)

If frozen, thaw the reagents on ice.

1. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.

2. Dilute the TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control:

   a. Pipet 196 µL of TE Buffer into a microcentrifuge tube, then add 4 µL of TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control. Mix well, then centrifuge briefly.

   b. Pipet 196.5 µL of TE Buffer into a second microcentrifuge tube, then add 8.5 µL of the dilution created in substep 2a. Mix well, then centrifuge briefly.

3. Prepare the reaction mix on ice:
   a. For each run, combine the following components sufficient for the number of DNA samples to be tested plus one positive control and one negative control.

All volumes include 10% overage.

IMPORTANT! The volumes in this table assume that you extracted sample DNA using an original sample input volume of 400 µL.

Component	Volume per DNA sample or control	Volume for *n* DNA samples plus 2 controls	Volume for 94 DNA samples plus 2 controls
TaqPath™ BactoPure™ Microbial Detection Master Mix (No ROX™) (2X)	10 µL	11 x (*n* + 2) µL	1056.0 µL
TaqPath™ Monkeypox/Orthopox Virus Multiplex Assay	1 µL	1.1 x (*n* + 2) µL	105.6 µL
Total reaction mix volume	**11 µL**	**—**	**1,161.6 µL**

4. Set up the reaction plate on ice:
   a. Pipet 11 µL of the reaction mix prepared in step 3 into each well of a MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL or a MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 0.2 mL.

      For other reaction plates that can be used, see “Required materials not supplied” on page 8.

   b. Gently vortex the sealed plate containing the purified sample DNA and negative control from the DNA extraction procedure, then centrifuge briefly to collect liquid at the bottom of the plate.

   c. Unseal the plate containing the purified sample DNA and negative control from the DNA extraction procedure. Add either sample DNA, negative control, or positive control to each well of the reaction plate, according to Table 3.

   d. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.

IMPORTANT!

- Use ONLY MicroAmp™ Optical Adhesive Film (Cat. No. 4311971, 4360954).
- DO NOT use optical caps, MicroAmp™ Clear Adhesive Film (Cat. No. 4306311), or any other film or sealing method.
- DO NOT heat seal the plate.
- When applying the MicroAmp™ Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and evaporation during PCR.

e. Vortex the plate at the highest setting speed with medium pressure for 5 seconds in the center, 5 seconds in each corner, and 5 seconds in the center again. Rotate the plate to ensure equal contact on the vortex mixer platform.

IMPORTANT! Vortex for a total of 30 seconds to ensure proper mixing. Failure to do so might result in incorrect (or inaccurate) clinical calls.

f. Centrifuge the reaction plate for 1–2 minutes at ≥650 × *g* (≥650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

IMPORTANT! Centrifuge the plate for 1–2 minutes to ensure bubbles are removed. Failure to do so might result in incorrect (or inaccurate) clinical calls.

Table 3 Reaction plate volumes

Component	Volume per reaction		
	DNA sample	Positive control	Negative control
Reaction mix (from step 3)	11 µL	11 µL	11 µL
Purified sample DNA (from DNA extraction)	9 µL	—	—
Positive control (diluted TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control from step 2)	—	9 µL	—
Negative control (from DNA extraction)	—	—	9 µL
Total volume	**20 µL**	**20 µL**	**20 µL**

IMPORTANT!

- Keep the real-time PCR reaction plate on ice or a cold block until it is loaded into the real-time PCR instrument.
- Run the real-time PCR reaction plate immediately after preparation. Failure to do so could result in degraded samples.

Prepare the RT-PCR reactions (384-well reaction plate)

If frozen, thaw the reagents on ice.

1. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.

2. Dilute the TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control:
   a. Pipet 196 µL of TE Buffer into a microcentrifuge tube, then add 4 µL of TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control. Mix well, then centrifuge briefly.

   b. Pipet 196.5 µL of TE Buffer into a second microcentrifuge tube, then add 8.5 µL of the dilution created in substep 2a. Mix well, then centrifuge briefly.

3. Prepare the reaction mix on ice.
   a. For each run, combine the following components sufficient for the number of DNA samples, plus one positive control per 384-well real-time PCR reaction plate, and one negative control from each extraction run.

      For example, if DNA samples from 4 extraction runs are combined on one 384-well real-time PCR plate, then 4 negative control wells (one from each extraction plate) need to be run on that 384-well real-time PCR plate.

All volumes include 10% overage.

IMPORTANT! The volumes in this table assume that you extracted sample DNA using an original sample input volume of 400 µL.

Component	Volume per DNA sample or control	Volume for *n* DNA samples plus 5 controls	Volume for 379 DNA samples plus 5 controls
TaqPath™ BactoPure™ Microbial Detection Master Mix (No ROX™) (2X)	10 µL	11 x (*n* + 5) µL	4,224 µL
TaqPath™ Monkeypox/Orthopox Virus Multiplex Assay	1 µL	1.1 x (*n* + 5) µL	422.4 µL
Total reaction mix volume	**11 µL**	**—**	**4,646.4 µL**

4. Set up the reaction plate on ice.
   a. Pipet 11 µL of the reaction mix prepared in step 3 into each well of a MicroAmp™ Optical 384-Well Reaction Plate with Barcode.

      For other reaction plates that can be used, see “Required materials not supplied” on page 8.

   b. Gently vortex the sealed plate containing the purified sample DNA and negative control from the DNA extraction procedure, then centrifuge briefly to collect liquid at the bottom of the plate.

   c. Unseal the plate containing the purified sample DNA and negative control from the DNA extraction procedure. Add either sample DNA, negative control, or positive control to each well of the reaction plate according to Table 4.

      IMPORTANT! To prevent sample contamination, unseal one extraction plate at a time, then reseal it after adding the samples to the real-time PCR reaction plate.

d. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.

IMPORTANT!

- Use ONLY MicroAmp™ Optical Adhesive Film (Cat. No. 4311971, 4360954).
- DO NOT use optical caps, MicroAmp™ Clear Adhesive Film (Cat. No. 4306311), or any other film or sealing method.
- DO NOT heat seal the plate.
- When applying the MicroAmp™ Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and evaporation during PCR.

e. Vortex the plate at the highest setting speed with medium pressure for 5 seconds in the center, 5 seconds in each corner, and 5 seconds in the center again. Rotate the plate to ensure equal contact on the vortex mixer platform.

IMPORTANT! Vortex for a total of 30 seconds to ensure proper mixing. Failure to do so might result in incorrect (or inaccurate) clinical calls.

f. Centrifuge the reaction plate for 1–2 minutes at ≥650 × *g* (≥650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

IMPORTANT! Centrifuge the plate for 1–2 minutes to ensure bubbles are removed. Failure to do so might result in incorrect (or inaccurate) clinical calls.

Table 4 Reaction plate volumes

Component	Volume per reaction		
	DNA sample	Positive control	Negative control
Reaction mix (from step 3)	11 µL	11 µL	11 µL
Purified sample DNA (from DNA extraction)	9 µL	—	—
Positive control (diluted TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control from step 2)	—	9 µL	—
Negative control (from DNA extraction)	—	—	9 µL
Total volume	**20.0 µL**	**20.0 µL**	**20.0 µL**

IMPORTANT!

- Keep the real-time PCR reaction plate on ice or a cold block until it is loaded into the real-time PCR instrument.
- Run the real-time PCR reaction plate immediately after preparation. Failure to do so could result in degraded samples.

4 Perform real-time PCR using the QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block)

Dye calibration for the QuantStudio™ 5 Real-Time PCR Instrument

Ensure the system calibrations are current. Refer to the standard calibration process in the instrument user guide.

Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument

The template (EDT) file contains the settings for the instrument run.

It is in the same compressed folder as the Pathogen Interpretive Software. The folder is `MPXOPX_EUA_QS596_0.1mL_AssayPanel_Template`.

The template must be transferred via a USB drive or other method to the computer on which QuantStudio™ Design and Analysis Desktop Software is installed.

IMPORTANT! Be careful to select the appropriate template file for your instrument and block type. Failure to do so can cause errors in the analysis.

1. After extracting the files from the compressed folder, select the following EDT file:

   `MPX OPX Template QS5 0_1ml da1_5_2 v1_0.edt`

   Note: The same template file is used for QuantStudio™ Design and Analysis Desktop Software v1.5.1 and v1.5.2.

2. Transfer the EDT file to the computer with QuantStudio™ Design and Analysis Desktop Software, using a USB drive or other method.

Set up and run the QuantStudio™ 5 Real-Time PCR Instrument

For more information about the instrument, see the documents listed in “Related documentation” on page 61.

Note: For the QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.1-mL block, use the system defaults for the PCR filters.

1. In the QuantStudio™ Design and Analysis Desktop Software home screen, in the **New Experiment** box, select **Create New Experiment ▸ Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (EDT) file for the QuantStudio™ 5 Real-Time PCR Instrument” on page 26.

   IMPORTANT! Be careful to select the appropriate template file for your instrument and block type. Failure to do so can cause errors in the analysis.
3. In the **Properties** tab, enter or confirm the following.
   - **Name**: Enter a unique name
   - **Instrument type**: **QuantStudio™ 5 System**
   - **Block type**: **96-Well 0.1-mL Block**
   - **Experiment type**: **Standard Curve**
   - **Chemistry**: **TaqMan™ Reagents**
   - **Run Mode**: **Standard**
4. In the **Method** tab, confirm that the **Volume** is **20 µL**, then confirm the thermal cycling protocol.

Step	Temperature[1]	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	10 seconds	40
Anneal / extend	60°C	30 seconds	

[1] Confirm that the ramp up rate is 2.74°C per second and the ramp down rate is 2.12°C per second for each step.

5. In the **Plate** screen, click **Quick Setup**.
6. In the **Plate Attributes** pane, confirm that the **Passive Reference** is set to **None**.
7. In the **Plate** tab, click **Advanced Setup**.

8. In the **Targets** table, confirm that the targets, reporter dyes, and quencher are listed correctly.

Target name[1]	Reporter dye	Quencher
Monkeypox virus	FAM	**None**
Orthopoxvirus	VIC	
RNase P	JUN	

[1] Orthopoxvirus refers to "non-variola *Orthopoxvirus*" for the TaqPath™ Monkeypox/Orthopox Virus DNA Kit.

IMPORTANT! Target names are case-sensitive and must be named as described.

9. Confirm that the targets above are assigned to each well in the plate layout.

10. In the plate layout pane, confirm the labeling of the control wells.
    - The template has one positive control (PC) and one negative control (NC) assigned to wells for reference.
    - The positive control must be named ***PC***. If additional characters are included, it must be named ***PC<>***, where <> is defined by the user, for example ***PC1***.
    - The negative control must be named ***NC***. If additional characters are included, it must be named ***NC<>***, where <> is defined by the user, for example ***NC1***.
    - Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

IMPORTANT! The positive and negative controls must be named as described.

11. For all targets in the positive control well, confirm that **Task** is set to **U (Unknown)**.

12. For all targets in the negative control well, confirm that **Task** is set to **U (Unknown)**.

13. In the **Samples** table, click **Add** to define the sample names. Create a unique sample name for each well in the physical plate.

IMPORTANT! Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act)

14. To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table.

    For all targets in the patient sample wells, confirm that **Task** is set to **U (Unknown)**.

Note: Wells that do not have a sample name will not be analyzed by the software.

15. Load the prepared and sealed real-time PCR reaction plate into the real-time PCR instrument.

16. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.

17. Enter a file name in the dialog box that prompts you to save the run file, then save the file.

5 Perform real-time PCR using the QuantStudio™ 5 Dx Real-Time PCR Instrument (96-well, 0.2-mL block)

Dye calibration for the QuantStudio™ 5 Dx Real-Time PCR Instrument

Ensure the system calibrations are current. Refer to the standard calibration process in the instrument user guide.

Transfer the template (EDT) file for the QuantStudio™ 5 Dx Real-Time PCR Instrument (QuantStudio™ 5 Dx IVD Software)

The template (EDT) file contains the settings for the instrument run.

It is in the same compressed folder as the Pathogen Interpretive Software. The folder is `MPXOPX_EUA_QS5Dx_AssayPanel_Template`.

The template must be transferred via a USB drive or other method to the computer on which the instrument with the QuantStudio™ 5 Dx IVD Software is installed.

IMPORTANT! Be careful to select the appropriate template file for your instrument and block type. Failure to do so can cause errors in the analysis.

1. After extracting the files from the compressed folder, select the following EDT file:

   `MPX OPX Template QS5 Dx da1_2_0 v1_0.edt`

2. Transfer the EDT file to the computer with the QuantStudio™ 5 Dx IVD Software, using a USB drive or other method.

The template must be installed in the QuantStudio™ 5 Dx IVD Software before the run is started. See "Install the template file in the QuantStudio™ 5 Dx IVD Software" on page 30.

Install the template file in the QuantStudio™ 5 Dx IVD Software

To install a template, the logged-in user must have the SAE permission of **Manage Installed Templates**. For information about SAE permissions, see the *QuantStudio™ 5 Dx IVD Software User Guide* (Pub. No. 100049556).

1. Open the QuantStudio™ 5 Dx IVD Software.
2. When prompted, log in with a user account with the appropriate permissions.
3. In the menu bar, select **Tools** ▸ **Template Menu**.
4. Click **Install**, then click **Yes** to confirm the **Template Installation Agreement**.
5. Navigate to the desired published template (EDT file), then click **Open**.
   The template is now installed and accessible in the **Template Menu**.
6. Click **Close**.

Set up and run the QuantStudio™ 5 Dx Real-Time PCR Instrument (QuantStudio™ 5 Dx IVD Software)

For more information about the QuantStudio™ 5 Dx Real-Time PCR Instrument, see the documents listed in "Related documentation" on page 61.

Note: For the QuantStudio™ 5 Dx Real-Time PCR Instrument, 96-well, 0.2-mL block, use the system defaults for the PCR filters.

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1. In the QuantStudio™ 5 Dx IVD Software, in the **New Experiment** box, select **Experiment Setup**.
2. Select the template file that you installed in "Install the template file in the QuantStudio™ 5 Dx IVD Software" on page 30.
3. Click **Create New Experiment**.
4. In the **Properties** tab, enter or confirm the following.
   - **Name**: Enter a unique name
   - **Instrument type**: **QuantStudio™ 5 Dx System**
   - **Block type**: **96-Well 0.2-mL Block**
   - **Experiment type**: **Standard Curve**
   - **Chemistry**: **TaqMan™ Reagents**

- **Run Mode**: **Standard**

5. In the **Method** tab, confirm that the **Volume** is **20 µL**, then confirm the thermal cycling protocol.

Step	Temperature[1]	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	10 seconds	40
Anneal / extend	60°C	30 seconds	

[1] Confirm that the ramp up rate is 2.74°C per second and the ramp down rate is 2.12°C per second for each step.

6. In the **Plate** screen, click **Quick Setup**.

7. In the **Plate Attributes** pane, confirm that the **Passive Reference** is set to **None**.

8. In the **Plate** tab, click **Advanced Setup**.

9. In the **Targets** table, confirm that the targets, reporter dyes, and quencher are listed correctly.

Target name[1]	Reporter dye	Quencher
Monkeypox Virus	FAM	**None**
Orthopoxvirus	VIC	
RNase P	JUN	

[1] Orthopoxvirus refers to "non-variola *Orthopoxvirus*" for the TaqPath™ Monkeypox/Orthopox Virus DNA Kit.

IMPORTANT! Target names are case-sensitive and must be named as described.

10. Confirm that the targets above are assigned to each well in the plate layout.

11. In the plate layout pane, confirm the labeling of the control wells.
    - The template has one positive control (PC) and one negative control (NC) assigned to wells for reference.
    - The positive control must be named ***PC***. If additional characters are included, it must be named ***PC***<>, where <> is defined by the user, for example ***PC1***.
    - The negative control must be named ***NC***. If additional characters are included, it must be named ***NC***<>, where <> is defined by the user, for example ***NC1***.
    - Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

IMPORTANT! The positive and negative controls must be named as described.

12. For all targets in the positive control well, confirm that **Task** is set to **U (Unknown)** for all of the targets.

13. For all targets in the negative control well, confirm that **Task** is set to **U (Unknown)** for all of the targets.

14. In **Samples** table, click **Add** to define the sample names. Create a unique sample name for each well in the physical plate.

    IMPORTANT! Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act).

15. To assign a sample to a well, select the well in the plate layout, then select sample from the **Samples** table.

    For all targets in the patient sample wells, confirm that **Task** is set to **U (Unknown)** for all the targets.

    Note: Wells that do not have a sample name will not be analyzed by the software.

16. Load the prepared and sealed real-time PCR reaction plate into the real-time PCR instrument.

17. Place a MicroAmp™ Optical Film Compression Pad gray side down on the surface of the real-time PCR reaction plate, to ensure a proper seal between the thermal cycler and the adhesive film.

    IMPORTANT!
    - Be careful to place the compression pad with the brown side up and the gray side down, centered on top of the plate.
    - Ensure the compression pad is free from wrinkles and signs of deterioration prior to use.

18. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.

    The **Enter Reason for Change** dialog box will appear.

19. In the **Enter Reason for Change** dialog box, click **OK**.

20. Enter a file name in the dialog box that prompts you to save the run file, then save the file.

21. If you are using a MicroAmp™ Optical Film Compression Pad, at the end of the run, remove the pad from the plate and store the pad inside the pack.

    IMPORTANT!
    - If the compression pad becomes stuck inside the thermal cycler, call service to clean the heated cover.
    - Between each use, place the pad back in the pouch so that it does not dry out.
    - Each compression pad may be used up to 20 times before discarding. Do not use more than 20 times.
    - Do NOT use the pad with other instruments, unless expressly instructed to do so in the user documentation.

6 Perform real-time PCR using the QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

Dye calibration for the QuantStudio™ 7 Flex Real-Time PCR Instrument

Ensure the system calibrations are current. In addition, JUN™ dye must be calibrated for use with this kit. Refer to the custom calibration process in the instrument user guide.

Transfer the template (EDT) file for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384–well block)

The template (EDT) file contains the settings for the instrument run.

It is in the same compressed folder as the Pathogen Interpretive Software. The folder is `MPXOPX_EUA_QS7384_AssayPanel_Template`.

The template must be transferred via a USB drive or other method to the computer on which the QuantStudio™ Real-Time PCR Software is installed.

IMPORTANT! Be careful to select the appropriate template file for your instrument and block type. Failure to do so can cause errors in the analysis.

1. After extracting the files from the compressed folder, select the following EDT file:

   `MPX OPX Template QS7 384 1_3 v1_0.edt`

2. Transfer the EDT file to the computer with QuantStudio™ Real-Time PCR Software, using a USB drive or other method.

Set up and run the QuantStudio™ 7 Flex Real-Time PCR Instrument

For more information about the instrument, see the documents listed in “Related documentation” on page 61.

1. In the QuantStudio™ Real-Time PCR Software home screen, click **Template**.
2. Browse to, then open the EDT file that you transferred in “Transfer the template (EDT) file for the QuantStudio™ 7 Flex Real-Time PCR Instrument (384–well block)” on page 33.

   IMPORTANT! Be careful to select the appropriate template file for your instrument, block type, and assay type. Failure to do so can cause errors in the analysis.

3. In the **Experiment Properties** tab, enter or confirm the following.
   - **Experiment Name**: Enter a unique name
   - **Instrument type**: **QuantStudio™ 7 Flex System**
   - **Block**: **384-well**
   - **Type of Experiment**: **Standard Curve**
   - **Reagents**: **TaqMan™ Reagents**
   - **Properties**: **Standard**
4. In the **Define** tab, in the **Targets** pane, confirm that the targets, reporter dyes, and quenchers are listed correctly.

Target name[1]	Reporter dye	Quencher
Monkeypox virus	FAM	**None**
Orthopoxvirus	VIC	
RNase P	JUN	

[1] Orthopoxvirus refers to "non-variola *Orthopoxvirus*" for the TaqPath™ Monkeypox/Orthopox Virus DNA Kit.

   IMPORTANT! Target names are case-sensitive and must be named as described.

5. In the **Define** tab, in the **Samples** pane, define a sample name for each sample. Create a unique sample name for each well in the physical plate.

   Note: Wells that do not have a sample name will not be analyzed by the software.

   IMPORTANT! Encrypt, pseudonymize, or anonymize personal data where possible following the requirements of HIPAA (Health Insurance Portability and Accountability Act)

6. In the **Define** screen, confirm that the **Passive Reference** is set to **None**.
7. In the **Assign** screen, confirm that targets are assigned to each well in the **Plate Layout** tab.

   To assign a target to a well, select the well in the plate layout, then select the targets from the **Targets** table.

8. In the **Assign** screen, in the **Plate Layout** tab, confirm the labeling of the control wells.
   - The template has one positive control and one negative control assigned to wells for reference.
   - The positive control must be named ***PC***. If additional characters are included, it must be named ***PC***<>, where <> is defined by the user, for example ***PC1***.
   - The negative control must be named ***NC***. If additional characters are included, it must be named ***NC***<>, where <> is defined by the user, for example ***NC1***.
   - Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.

   IMPORTANT! The positive and negative controls must be named as described.

9. In the **Assign** screen, confirm the **Task** assignments.
   - For wells with a positive control (***PC***), confirm that the **Task** is set to **U (Unknown)** for all of the targets.
   - For wells with a negative control (***NC***), confirm that the **Task** is set to **U (Unknown)** for all of the targets.
   - For the wells with a patient sample, confirm that the **Task** is set to **U (Unknown)** for all of the targets.

10. In the **Assign** screen, assign a sample name to each well to match the physical plate.

    To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table.

    Note: Wells that do not have a sample name will not be analyzed by the software.

11. In the **Run Method** tab, confirm that the **Reaction Volume Per Well** is 20 µL, then confirm the thermal protocol.

Step	Temperature[1]	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	10 seconds	40
Anneal / extend	60°C	30 seconds	

[1] Confirm that the ramp rate for each step is 1.6°C per second.

12. In the **Run Method** screen, select **Optical Filters**, then confirm that the 8 PCR filters shown in Table 5 are selected.

    To enable **Optical Filters**, navigate to **Tools** ▸ **Preferences**, then in the **Defaults** tab, select **Show optical filters for run method**.

Table 5 PCR Filters

		Emission Filter					
		m1(520±15)	m2(558±11)	m3(586±10)	m4(623±14)	m5(682±14)	m6(711±12)
Excitation Filter	x1(470±15)	✔	✔				
	x2(520±10)		✔	✔			
	x3(550±11)			✔	✔		
	x4(580±10)				✔		
	x5(640±10)					✔	
	x6(662±10)						

13. Load the prepared and sealed real-time PCR reaction plate into the real-time PCR instrument.

14. In the **Run** screen, click **Start Run**, then select your instrument from the drop-down list.

15. Enter a file name in the dialog box that prompts you to save the run file, then save the file.

7 Analysis and results

Software and instrument compatibility

Software	Description
Pathogen Interpretive Software v1.1	Data analysis and results interpretation software
SAE Administrator Console Dx v1.0 or SAE Administrator Console Dx v1.2	Security and audit tool
Pathogen Interpretive Software 1.0.0 DAT (SAE Profile)	Provides settings to connect the SAE Administrator Console Dx with the Pathogen Interpretive Software
MPXOPX-EUA_QS5-9601_1.0.0.zip	Assay panel for QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block)
MPXOPX-EUA_QS5Dx-9602_1.0.0.zip	Assay panel for QuantStudio™ 5 Dx Real-Time PCR Instrument
MPXOPX-EUA_QS7F-384_1.0.0.zip	Assay panel for QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)

For information on how to obtain the software, see “Obtain the software package” on page 38.

The following table lists the assay panel that is compatible with the Pathogen Interpretive Software v1.1, your instrument, and its associated analysis software.

To obtain the correct analysis software or firmware version for your real-time PCR instrument, go to **thermofisher.com/validatedfirmware**, then select your instrument in the **Real-Time PCR** section. If you have questions or problems finding the correct version, contact technical support (see Appendix D, “Documentation and support”).

Instrument	Data collection software used with the instrument	Pathogen Interpretive Software	Assay panel file name[1]
QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block)	QuantStudio™ Design and Analysis Desktop Software v1.5.1 or v1.5.2	v1.1	MPXOPX-EUA_QS5-9601_1.0.0.zip
QuantStudio™ 5 Dx Real-Time PCR Instrument (96-well, 0.2-mL block)	QuantStudio™ 5 Dx IVD Software v1.2.0	v1.1	MPXOPX-EUA_QS5Dx-9602_1.0.0.zip
QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)	QuantStudio™ Real-Time PCR Software v1.3	v1.1	MPXOPX-EUA_QS7F-384_1.0.0.zip

[1] For information on how to install the assay panel, see “Install the assay panel” on page 39.

Obtain the software package

To perform data analysis and results interpretation, you must use the following software:

- Pathogen Interpretive Software v1.1
- SAE Administrator Console Dx v1.0 or SAE Administrator Console Dx v1.2
- Pathogen Interpretive Software 1.0.0 DAT (SAE Profile)
- The appropriate assay panel for your instrument:
  - MPXOPX-EUA_QS5-9601_1.0.0.zip
  - MPXOPX-EUA_QS5Dx-9602_1.0.0.zip
  - MPXOPX-EUA_QS7F-384_1.0.0.zip

For installation and configuration instructions, see Appendix D, “Documentation and support”.

The software and assay panel can be installed on a customer-provided computer with the following minimum computer system specifications:

- Operating system—Windows™ 10 (64-bit), with language set to English
- Processor—Pentium® 4 or higher
- Memory—8 GB RAM minimum
- Hard drive—10 GB minimum free space
- Monitor—1280 × 1024 resolution or higher

Note: Do not install the Pathogen Interpretive Software and SAE Administrator Console Dx if it is already installed on your computer. The software can be used with several assay panels.

To obtain the software, contact the instrument service team. Go to **thermofisher.com/contactus**.

Install the assay panel

An assay panel contains the analysis settings that are used for data analysis in the Pathogen Interpretive Software.

The assay panel is located in the same compressed folder as the software and is instrument-specific. Select the folder for your instrument.

Note: The software must be installed before installing the assay panel.

Only one matching assay panel is required to analyze a data file.

1. Extract the files from the compressed folder for your instrument.

Instrument	Folder name
QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block)	`MPXOPX_EUA_QS596_0.1mL_AssayPanel_Template`
QuantStudio™ 5 Dx Real-Time PCR Instrument (96-well, 0.2-mL block)	`MPXOPX_EUA_QS5Dx_AssayPanel_Template`
QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)	`MPXOPX_EUA_QS7384_AssayPanel_Template`

2. In the software, click **System** ▸ **Assay Panels** ▸ **Actions** ▸ **Install**.

3. Navigate to, then open the assay panel for your instrument.

Instrument	Assay panel file name
QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block)	MPXOPX-EUA_QS5-9601_1.0.0.zip
QuantStudio™ 5 Dx Real-Time PCR Instrument (96-well, 0.2-mL block)	MPXOPX-EUA_QS5Dx-9602_1.0.0.zip
QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)	MPXOPX-EUA_QS7F-384_1.0.0.zip

4. Click **Install**.

   Note: If the assay panel is already installed, you will be prompted to confirm the assay panel upgrade.

   The assay panel is added to the **Assay Panels** library.

For more information about assay panels, including uninstalling assay panels, see the documentation for the Pathogen Interpretive Software (Appendix D, “Documentation and support”).

Analyze the data

For detailed instructions about using the software, click the **Help** menu in the Pathogen Interpretive Software.

1. Using a USB drive or other method, transfer the EDS files from the computer with the data collection software to the computer with the Pathogen Interpretive Software.
2. In the software screen, select one of the following options:

Option	Description
In the **Data Gallery**, click **Actions** ▸ **Open File**.	The data file will open in the current window.
In the **Data Gallery**, click **Actions** ▸ **Open File in New Window**.	The data file will open in a new window.

3. Navigate to, then open the data file.
   - The data file opens and the results are displayed in the **Presence Absence** tab.
   - The data file is added to the **Data Gallery**.

Note: If the data file has already been added to the **Data Gallery**, click the file to open the file in the current window or hover over the file, then click ··· **(Actions)** ▸ **Open in new window** to open the file in a new window.

Interpretation of the results

Interpretation of the results is performed by the Pathogen Interpretive Software using one of the following panels:

Instrument	Assay panel file name
QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block)	MPXOPX-EUA_QS5-9601_1.0.0.zip
QuantStudio™ 5 Dx Real-Time PCR Instrument (96-well, 0.2-mL block)	MPXOPX-EUA_QS5Dx-9602_1.0.0.zip
QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block)	MPXOPX-EUA_QS7F-384_1.0.0.zip

For information about the C_t values that are used by the software to interpret results, see Appendix A, “Ct cutoff values for assay targets”.

Quality control and validity of results

A minimum of one negative control and one positive control must be present for each run. All control wells must pass for the real-time PCR plate to be considered valid (Table 6).

Negative control wells must be run for each extraction that is represented on a real-time PCR plate. All control wells must pass for the real-time PCR plate to be considered valid.

Validation of results is performed automatically by the software based on performance of the positive and negative controls.

Table 6 Control samples

Sample	Monkeypox virus	Non-variola *Orthopoxvirus*	RNase P	Call	Status	Assessment
Positive control	POS	POS	POS	Presence	Passed	REPORT
Negative control	NEG	NEG	NEG	Absence	Passed	REPORT
Positive control or negative control	All other scenarios			Invalid	Failed	RETEST[1]

[1] Retesting can be performed with a new aliquot of the same control.

Table 7 Results interpretation for viral targets in patient samples

Sample[1]			Call	Assessment
Monkeypox virus	Non-variola *Orthopoxvirus*	RNase P		
POS	POS	POS or NEG	Presence	REPORT - Monkeypox virus and non-variola *Orthopoxvirus* Detected
POS	NEG	POS or NEG	Presence	REPORT - Monkeypox virus Detected[2]
NEG	POS	POS or NEG	Presence	REPORT - non-variola *Orthopoxvirus* Detected, Monkeypox virus Not Detected[3]
NEG	NEG	POS	Absence	REPORT - Monkeypox virus and non-variola *Orthopoxvirus* Not Detected
NEG	NEG	NEG	Invalid	RETEST[4]

[1] Controls must pass for viral targets to be interpreted (Table 6).

[2] Low concentrations of DNA in a sample can result in Monkeypox virus DNA Detected and non-variola *Orthopoxvirus* Not Detected.

[3] Low concentrations of DNA in a sample can result in Monkeypox virus Not Detected and non-variola *Orthopoxvirus* Detected; this result can occur in the case of low monkeypox viral concentration. Monkeypox virus Not Detected and non-variola *Orthopoxvirus* Detected may also indicate another *Orthopoxvirus* infection although no other known orthopoxviruses are currently circulating in the United States. If clinically indicated, consider collecting another specimen.

[4] Retesting must be performed by re-extracting the DNA from the original sample and repeating the real-time PCR. If the repeat result remains invalid, consider collecting a new specimen.

8 Conditions of authorization for labs

The TaqPath™ Monkeypox/Orthopox Virus DNA Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: **fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-emergency-use-authorizations-medical-devices**.

To assist clinical laboratories running the TaqPath™ Monkeypox/Orthopox Virus DNA Kit, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories[2] that receive the TaqPath™ Monkeypox/Orthopox Virus DNA Kit must notify the relevant public health authorities of their intent to run the product prior to initiating testing.
- Authorized laboratories using the TaqPath™ Monkeypox/Orthopox Virus DNA Kit must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories using the TaqPath™ Monkeypox/Orthopox Virus DNA Kit must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the TaqPath™ Monkeypox/Orthopox Virus DNA Kit must use the TaqPath™ Monkeypox/Orthopox Virus DNA Kit as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents, and authorized materials required to use the TaqPath™ Monkeypox/Orthopox Virus DNA Kit are not permitted.
- Authorized laboratories must have a process in place to track adverse events and report to Life Technologies Corporation (a part of Thermo Fisher Scientific, Inc.) (**techservices@thermofisher.com**; 1 800 955 6288) and to FDA pursuant to 21 CFR Part 803.
- All laboratory personnel using the test must be appropriately trained in real-time PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Life Technologies Corporation (a part of Thermo Fisher Scientific, Inc.), its authorized distributors, and authorized laboratories must collect information on the performance of the TaqPath™ Monkeypox/Orthopox Virus DNA Kit and must report any significant deviations from the established performance characteristics of the TaqPath™ Monkeypox/Orthopox Virus DNA Kit of which they become aware to DMD/OHT7/OPEQ/CDRH (via email: **CDRH-EUA-Reporting@fda.hhs.gov**). In addition, authorized distributor(s) and authorized laboratories report to Life Technologies Corporation (a part of Thermo Fisher Scientific, Inc.) (**techservices@thermofisher.com**; 1 800 955 6288).

[2] For ease of reference, this document will refer to "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

- Life Technologies Corporation (a part of Thermo Fisher Scientific, Inc.), its authorized distributor(s), and authorized laboratories using the TaqPath™ Monkeypox/Orthopox Virus DNA Kit must ensure that any records associated with this EUA, are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

9 Performance characteristics

Analytical performance of the TaqPath™ Monkeypox/Orthopox Virus DNA Kit was evaluated as described in the following sections.

Limit of detection (LoD)

The LoD study established the lowest monkeypox (mpox) and non-variola orthopox viral concentrations (Genomic Copy Equivalents or GCE) that can be detected by the TaqPath™ Monkeypox/Orthopox Virus DNA Kit at least 95% of the time.

The LoD for each viral target was determined using contrived specimens prepared using leftover, pooled negative lesion swab specimen matrix in VTM spiked at various concentrations with synthetic monkeypox virus DNA and genomic vaccinia virus DNA (selected to represent orthopoxviruses). Individual lesion specimens used for this study were confirmed negative for monkeypox virus and non-variola *Orthopoxvirus* before pooling. Synthetic monkeypox virus DNA and genomic vaccinia virus DNA were quantitated using Droplet Digital PCR (ddPCR). Sample extraction was performed using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and each extraction replicate was tested on three PCR instruments: QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block), QuantStudio™ 5 Dx Real-Time PCR Instrument (96-well, 0.2-mL block), and QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block).

The study was conducted in three phases. Phase I consisted of finding an approximate concentration range for LoD. Phase II refined the concentration test range based on Phase I results. Phase III confirmed the LoD with 20 test replicates. The determined LoD for each assay target for the three claimed instruments using both spiked materials is presented in Table 8 and Table 9.

Table 8 Mpox/Non-variola *Orthopoxvirus* LoD determination using Twist Synthetic Control Material

Concentration (GCE/mL)		Hit rate (%) # detected/# tested			Mean C_t (SD[1])		
MPXV	OPXV[2]	MPXV	OPXV	RNase P	MXV	OPXV	RNase P
QuantStudio™ 5 Real-Time PCR Instrument							
100	200	100 (20/20)	100 (20/20)	100 (20/20)	35.24 (0.47)	34.16 (0.39)	27.79 (0.19)
50	100	70 (14/20)	95 (19/20)	100 (20/20)	36.48 (0.97)	35.55 (0.69)	27.79 (0.17)
QuantStudio™ 5 Dx Real-Time PCR Instrument							
100	200	100 (20/20)	100 (20/20)	100 (20/20)	35.81 (0.63)	34.84 (0.58)	28.06 (0.13)
50	100	65 (13/20)	100 (20/20)	100 (20/20)	36.64 (0.97)	35.57 (0.60)	27.99 (0.12)
QuantStudio™ 7 Flex Real-Time PCR Instrument							
100	200	95 (19/20)	100 (20/20)	100 (20/20)	36.30 (0.90)	35.25 (0.64)	28.20 (0.62)
50	100	85 (17/20)	95 (19/20)	100 (20/20)	37.15 (0.95)	36.33 (0.85)	28.31 (0.49)

[1] SD: Standard Deviation

[2] Twist synthetic control contains DNA fragments that are detected by both the MPXV assay and OPXV assay at a ratio of 1 to 2 based on sequence data. The concentrations of OPXV are calculated from the determined MPXV concentrations.

Table 9 Non-variola *Orthopoxvirus* LoD determination using Genomic Vaccinia DNA Material

Concentration (GCE/mL)	Hit rate (%) # detected/# tested			Mean C_t (SD[1])		
	MPXV	OPXV	RNase P	MXV	OPXV	RNase P
QuantStudio™ 5 Real-Time PCR Instrument						
300	0	100 (20/20)	100 (20/20)	N/A	34.90 (0.56)	27.64 (0.19)
250	0	100 (20/20)	100 (20/20)	N/A	35.43 (0.53)	27.67 (0.10)
QuantStudio™ 5 Dx Real-Time PCR Instrument						
300	0	95 (19/20)	100 (20/20)	N/A	35.40 (0.74)	27.96 (0.10)
250	0	100 (20/20)	100 (20/20)	N/A	35.82 (0.61)	28.00 (0.09)
QuantStudio™ 7 Flex Real-Time PCR Instrument						
300	0	100 (20/20)	100 (20/20)	N/A	36.07 (0.67)	28.03 (0.38)
250	0	100 (20/20)	100 (20/20)	N/A	36.13 (0.65)	28.04 (0.28)

[1] SD: Standard Deviation

The LoD for the MPXV target using the Twist material is 100 GCE/mL for the three claimed RT-PCR instruments. The LoD for the OPXV target using the Twist material is 100 GCE/mL for the three claimed RT-PCR instruments. Based on the testing algorithm, the overall assay LoD established with the Twist material is 100 GCE/mL for all instruments. When using the genomic vaccinia DNA, the LoD of the OPXV target and overall assay LoD was 250 GCE/mL for the three claimed RT-PCR instruments.

Reactivity (Inclusivity)

In silico analysis was performed to determine the predicted reactivity (inclusivity) of the TaqPath™ Monkeypox/Orthopox Virus DNA Kit primer/probe sequences with all known strains/isolates of monkeypox virus (Clades I and II) and non-variola *Orthopoxvirus* sequences. The analysis used 1,886 near full-length monkeypox genomes from GISAID (1,850 clade II sequences, 32 clade I sequences, 4 unannotated) and 1,037 genomes from GenBank™ (taxon ID 10244) that had been deposited as of October 11, 2022. Based upon BLAST® analysis, the TaqPath™ Monkeypox/Orthopox Virus DNA Kit showed 100% homology to ≥99.6% of monkeypox strains in GISAID and GenBank™ databases.

The impacts of mismatched sequences were assessed and concluded to have minimal impact on the assays' ability to detect monkeypox virus strains, as mutation locations in assay primers and probes indicate that many of the observed mismatches shall not significantly impact strain detection and the predicted T_m values of assays to mismatched targets are higher than the annealing temperature.

The predicted inclusivity of the non-variola *Orthopoxvirus* target was evaluated using sequences of monkeypox, cowpox, camelpox, ectromelia, vaccinia, Akhmeta, buffalopox, horsepox, taterapox, orthopox abatino, and Alaskapox viruses as of November 8, 2022. Of the 1,037 monkeypox virus sequences that were evaluated, 1,037 demonstrated 100% homology to the OPXV primers/probe set. Overall, BLAST® analysis supports the predicted detection of all viral sequences analyzed, except for 2 Akhmeta virus sequences and 1 Alaskapox virus sequence due to multiple mismatches in the *Orthopoxvirus* forward primer and probe.

Cross-reactivity

An *in silico* study assessed the potential cross-reactivity of the TaqPath™ Monkeypox/Orthopox Virus DNA Kit primer/probe sequences and the organisms presented in Table 10, which include related viruses and potential co-infecting organisms.

The % homology of the primers and probes to the following organisms in NCBI GenBank™ was calculated.

Table 10 Organisms used for *in silico* cross-reactivity analysis

Type	Organism/strain
Bacteria	*Acinetobacter calcoaceticus* strain=EGD_AQ_BF14
	Bacteroides fragilis strain=BIOML-A91
	Chlamydia trachomatis strain=SC110
	Corynebacterium diphtheriae

Table 10 Organisms used for in silico cross-reactivity analysis *(continued)*

Type	Organism/strain
Bacteria	*Corynebacterium jeikeium* strain=Cj47453
	Enterococcus faecalis
	Escherichia coli strain=NCTC9120
	Lactobacillus crispatus strain=UMNLC2
	Mycoplasma genitalium strain=G37
	Mycoplasma pneumoniae strain=S91-tet-R
	Neisseria gonorrhoeae strain=NS3482
	Pseudomonas aeruginosa strain=PS2004
	Staphylococcus aureus strain=T020_N01_C07
	Staphylococcus epidermidis strain=JH-S-1
	Streptococcus agalactiae strain=JF4679
	Streptococcus dysgalactiae subsp. equisimilis (SDSE) strain=KNZ01 (group C Streptococcus)[1]
	Streptococcus dysgalactiae subsp. equisimilis (SDSE) strain=KNZ01 (group G Streptococcus)[1]
	Streptococcus mitis strain=BCC65
	Streptococcus pyogenes strain=NGAS227
	Treponema pallidum strain=Japan348
Fungi	*Candida albicans* SC5314
	Trichophyton rubrum strain CDCF0616
Protozoa	*Trichomonas vaginalis* G3
Virus	Herpes simplex virus (HSV-1 and HSV-2): Human herpesvirus 1 strain 17
	Herpes simplex virus (HSV-1 and HSV-2): Human herpesvirus 2 strain HG52
	Human papillomavirus (HPV): Human papillomavirus type 201, Gammapapillomavirus HPV127
	Molluscum contagiosum virus subtype 1
	Varicella-zoster virus (chickenpox): Human alphaherpesvirus 3 strain Ellen isolate ATCC VR-1367 clone Ellen
	Variola virus (smallpox)
	Cowpox virus[2]
	Camelpox virus[2]
	Ectromelia (mousepox) virus[2]

Table 10 Organisms used for in silico cross-reactivity analysis *(continued)*

Type	Organism/strain
Virus	Vaccinia virus[2]
—	Human genomic DNA

[1] *Streptococcus dysgalactiae subsp. equisimilis* (SDSE) belongs to both groups C and G streptococci.

[2] Off target to monkeypox virus assay, but not the non-variola *Orthopoxvirus* assay.

Results demonstrated that for the genomic sequences of the microorganisms evaluated in Table 10, cross-reactivity is not predicted for the MPXV and OPXV primer/probe sets included in the TaqPath™ Monkeypox/Orthopox Virus DNA Kit.

Transport media equivalency

A study was performed to determine if the TaqPath™ Monkeypox/Orthopox Virus DNA Kit performed equivalently when using lesion swab samples collected in UTM and VTM. Contrived samples were prepared by spiking in quantified synthetic DNA for monkeypox virus and genomic DNA from vaccinia virus into pooled negative lesion samples collected in UTM and pooled negative lesion samples collected in VTM at 2X and 5X LoD concentrations. Each sample was extracted with the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and tested on the QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.1-mL block), QuantStudio™ 5 Dx Real-Time PCR Instrument (96-well, 0.2-mL block), and QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well block).100% concordance to the expected results was observed, as shown in Table 11.

Table 11 Transport media equivalency results summary

Instrument	Conc. (LoD)	UTM			VTM		
		MPXV avg. C_t (# detected/# tested)	OPXV avg. C_t (# detected/# tested)	RNase P (# detected/# tested)	MPXV avg. C_t (# detected/# tested)	OPXV avg. C_t (# detected/# tested)	RNase P (# detected/# tested)
Twist Synthetic Control							
QuantStudio™ 5 Real-Time PCR Instrument	5X LoD	33.07 (10/10)	31.98 (10/10)	29.39 (10/10)	33.28 (10/10)	32.23 (10/10)	24.32 (10/10)
	2X LoD	34.62 (40/40)	33.48 (40/40)	29.73 (40/40)	34.54 (40/40)	33.48 (40/40)	24.73 (40/40)
	0X LoD	UND[1] (0/10)	UND (0/10)	29.06 (10/10)	UND (0/10)	UND (0/10)	24.04 (10/10)
QuantStudio™ 5 Dx Real-Time PCR Instrument	5X LoD	33.23 (10/10)	32.32 (10/10)	29.56 (10/10)	33.59 (10/10)	32.38 (10/10)	24.40 (10/10)
	2X LoD	35.06 (40/40)	33.93 (40/40)	30.07 (40/40)	34.85 (40/40)	33.85 (40/40)	24.97 (40/40)
	0X LoD	UND (0/10)	UND (0/10)	29.53 (10/10)	UND (0/10)	UND (0/10)	24.42 (10/10)
QuantStudio™ 7 Flex Real-Time PCR Instrument	5X LoD	34.36 (10/10)	33.29 (10/10)	30.20 (10/10)	34.11 (10/10)	33.20 (10/10)	24.86 (10/10)
	2X LoD	35.50 (40/40)	34.36 (40/40)	30.21 (40/40)	35.39 (40/40)	34.30 (40/40)	25.06 (40/40)
	0X LoD	UND (0/10)	UND (0/10)	29.31 (10/10)	UND (0/10)	UND (0/10)	24.34 (10/10)
Vaccinia Genomic DNA							
QuantStudio™ 5 Real-Time PCR Instrument	5X LoD	UND (0/10)	32.73 (10/10)	29.18 (10/10)	UND (0/10)	33.42 (10/10)	24.56 (10/10)
	2X LoD	UND (0/40)	34.37 (40/40)	29.30 (40/40)	UND (0/40)	34.62 (40/40)	24.32 (40/40)
	0X LoD	UND (0/10)	UND (0/10)	29.01 (10/10)	UND (0/10)	UND (0/10)	24.25 (10/10)
QuantStudio™ 5 Dx Real-Time PCR Instrument	5X LoD	UND (0/10)	33.12 (10/10)	29.51 (10/10)	UND (0/10)	33.62 (10/10)	24.76 (10/10)
	2X LoD	UND (0/40)	34.63 (40/40)	29.58 (40/40)	UND (0/40)	34.84 (40/40)	24.58 (40/40)
	0X LoD	UND (0/10)	UND (0/10)	29.40 (10/10)	UND (0/10)	UND (0/10)	24.72 (10/10)
QuantStudio™ 7 Flex Real-Time PCR Instrument	5X LoD	UND (0/10)	33.95 (10/10)	29.98 (10/10)	UND (0/10)	34.45 (10/10)	25.10 (10/10)

Table 11 Transport media equivalency results summary *(continued)*

Instrument	Conc. (LoD)	UTM			VTM		
		MPXV avg. C_t (# detected/# tested)	OPXV avg. C_t (# detected/# tested)	RNase P (# detected/# tested)	MPXV avg. C_t (# detected/# tested)	OPXV avg. C_t (# detected/# tested)	RNase P (# detected/# tested)
QuantStudio™ 7 Flex Real-Time PCR Instrument	2X LoD	UND (0/40)	35.23 (40/40)	29.76 (40/40)	UND (0/40)	35.47 (40/40)	24.66 (40/40)
	0X LoD	UND (0/10)	UND (0/10)	29.17 (10/10)	UND (0/10)	UND (0/10)	24.52 (10/10)

[1] UND: Undetermined (Not Detected)

Specimen stability

A specimen stability study was performed to establish refrigerated storage claims for UTM. Individual lesion swab samples in UTM that were confirmed negative for monkeypox virus and non-variola *Orthopoxvirus* were pooled and spiked with Twist synthetic Control DNA at 150 GCE/mL (1.5X LoD for MPXV and 3X LoD for OPXV) and 300 GCE/mL (3X LoD for MPXV and 6X LoD for OPXV). The contrived samples were stored at 2–8°C for 2 hours, 3 days, and 7 days, then tested on the QuantStudio™ 5 Dx Real-Time PCR Instrument. Testing of samples following refrigerated storage conditions demonstrated 100% detection of all sample replicates, with no apparent degradation of target DNA. The recommended storage conditions for lesion swabs collected in UTM is refrigerated temperature (2–8°C) for up to 7 days.

For lesion swab specimens collected in VTM, refer to the CDC website for recommended storage and shipping information[3]. Lesion swab specimens in VTM can be stored at 5°C±3°C for a maximum of 7 days prior to testing or stored at –20°C or colder for a maximum of 30 days (with a maximum of 1 freeze/thaw) prior to testing.

[3] **https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html** (*Guidelines for Collecting and Handling Specimens for Mpox Testing | Monkeypox | Poxvirus | CDC*)

Clinical evaluation

A clinical performance evaluation was performed using 30 monkeypox virus contrived positive samples, 30 non-variola *Orthopoxvirus* contrived positive samples, and 30 negative clinical lesion samples using the TaqPath™ Monkeypox/Orthopox Virus DNA Kit. Contrived samples targeted 2X, 3X, and 5X LoD for monkeypox virus and non-variola *Orthopoxvirus*. Each contrived clinical specimen was prepared using an individual clinical matrix (i.e., leftover negative lesion swab specimen in UTM or VTM) spiked with synthetic monkeypox virus DNA or genomic vaccinia virus DNA (selected to represent orthopoxviruses). All 62 contrived positive clinical specimens (30 samples in UTM and 32 samples in VTM) were detected with both the MPXV and OPXV targets. Positive percent agreement (PPA) is 100% (95% CI: 94.17% to 100.00%). The negative percent agreement (NPA) is 100% (30/30, 95% CI: 88.65 to 100.00%), with all specimens negative for the MPXV and OPXV targets and positive for the RNase P target. There was one invalid result generated with a negative VTM specimen (RNase P>35) on all three RT-PCR instruments. An invalid patient sample is re-tested using extracted DNA from the same patient sample, if available. There was not sufficient VTM specimen matrix remaining, and therefore testing of an additional negative VTM specimen was used. The performance of contrived positive and negative specimens was the same when the TaqPath™ Monkeypox/Orthopox Virus DNA Kit was run on the QuantStudio™ 5 Real-Time PCR Instrument, QuantStudio™ 5 Dx Real-Time PCR Instrument, and QuantStudio™ 7 Flex Real-Time PCR Instrument. Contrived clinical testing results are summarized in Table 12–Table 14.

Table 12 Summary of contrived clinical study results generated on the QuantStudio™ 5 Real-Time PCR Instrument

Conc.	Total replicates	Transport media	# of samples	MPXV		OPXV		RNaseP	
				Mean C_t (SD[1])	Hit rate	Mean C_t (SD)	Hit rate	Mean C_t (SD)	Hit rate
Negative	30[2]	UTM	15	UND[3]	0/15	UND	0/15	29.04 (0.96)	15/15
		VTM	15	UND	0/15	UND	0/15	23.12 (2.57)	15/15
Twist Synthetic Control DNA									
2X LoD	21	UTM	15	35.15 (0.60)	15/15	33.96 (0.31)	15/15	28.53 (1.13)	15/15
		VTM	6	34.87 (0.78)	6/6	33.83 (0.20)	6/6	24.30 (5.05)	6/6
3X LoD	5	VTM	5	34.42 (0.37)	5/5	33.46 (0.53)	5/5	24.67 (2.74)	5/5
5X LoD	5	VTM	5	33.68 (0.66)	5/5	32.64 (0.40)	5/5	22.47 (2.99)	5/5
Genomic Vaccinia DNA									
2X LoD	21	UTM	15	UND	0/15	34.74 (0.77)	15/15	28.30 (0.93)	15/15

Table 12 Summary of contrived clinical study results generated on the QuantStudio 5 Real-Time PCR Instrument *(continued)*

Conc.	Total replicates	Transport media	# of samples	MPXV		OPXV		RNaseP	
				Mean C_t (SD[1])	Hit rate	Mean C_t (SD)	Hit rate	Mean C_t (SD)	Hit rate
2X LoD	21	VTM	6	UND	0/6	34.44 (0.47)	6/6	22.64 (1.19)	5/6
3X LoD	5	VTM	5	UND	0/5	34.07 (0.35)	5/5	24.77 (2.84)	5/5
5X LoD	5	VTM	5	UND	0/5	32.85 (0.57)	5/5	22.41 (3.04)	5/5

[1] SD: Standard Deviation
[2] One (1) negative specimen in VTM lacked a valid RNase P result and was replaced by an additional negative specimen in VTM.
[3] UND: Undetermined (Negative)

Table 13 Summary of contrived clinical study results generated on the QuantStudio™ 5 Dx Real-Time PCR Instrument

Conc.	Total replicates	Transport media	# of samples	MPXV		OPXV		RNaseP	
				Mean C_t (SD[1])	Hit rate	Mean C_t (SD)	Hit rate	Mean C_t (SD)	Hit rate
Negative	30[2]	UTM	15	UND[3]	0/15	UND	0/15	29.24 (0.95)	15/15
		VTM	15	UND	0/15	UND	0/15	23.46 (2.61)	15/15
Twist Synthetic Control DNA									
2X LoD	21	UTM	15	35.24 (0.46)	15/15	34.13 (0.37)	15/15	28.77 (1.01)	15/15
		VTM	6	35.55 (0.75)	6/6	34.47 (0.55)	6/6	22.57 (1.11)	5/6
3X LoD	5	VTM	5	34.43 (0.32)	5/5	33.81 (0.56)	5/5	25.00 (2.73)	5/5
5X LoD	5	VTM	5	33.82 (0.40)	5/5	33.00 (0.20)	5/5	22.73 (3.05)	5/5
Genomic Vaccinia DNA									
2X LoD	21	UTM	15	UND	0/15	34.92 (0.32)	15/15	28.58 (1.01)	15/15
		VTM	6	UND	0/6	35.11 (0.52)	6/6	22.73 (1.24)	5/6

Table 13 Summary of contrived clinical study results generated on the QuantStudio 5 Dx Real-Time PCR Instrument *(continued)*

Conc.	Total replicates	Transport media	# of samples	MPXV		OPXV		RNaseP	
				Mean C_t (SD[1])	Hit rate	Mean C_t (SD)	Hit rate	Mean C_t (SD)	Hit rate
3X LoD	5	VTM	5	UND	0/5	34.58 (0.87)	5/5	25.03 (2.80)	5/5
5X LoD	5	VTM	5	UND	0/5	33.26 (0.24)	5/5	22.74 (3.04)	5/5

[1] SD: Standard Deviation
[2] One (1) negative specimen in VTM lacked a valid RNase P result and was replaced by an additional negative specimen in VTM.
[3] UND: Undetermined (Negative)

Table 14 Summary of contrived clinical study results generated on the QuantStudio™ 7 Flex Real-Time PCR Instrument

Conc.	Total replicates	Transport media	# of samples	MPXV		OPXV		RNaseP	
				Mean C_t (SD[1])	Hit rate	Mean C_t (SD)	Hit rate	Mean C_t (SD)	Hit rate
Negative	30[2]	UTM	15	UND[3]	0/15	UND	0/15	29.44 (1.10)	15/15
		VTM	15	UND	0/15	UND	0/15	23.27 (2.57)	15/15
Twist Synthetic Control DNA									
2X LoD	21	UTM	15	35.65 (0.53)	15/15	34.74 (0.29)	15/15	28.89 (0.99)	15/15
		VTM	6	35.80 (0.85)	6/6	35.07 (0.93)	6/6	22.43 (1.51)	5/6
3X LoD	5	VTM	5	34.79 (0.70)	5/5	33.83 (0.93)	5/5	24.94 (2.58)	5/5
5X LoD	5	VTM	5	33.88 (0.24)	5/5	32.91 (0.56)	5/5	22.70 (2.76)	5/5
Genomic Vaccinia DNA									
2X LoD	21	UTM	15	UND	0/15	35.43 (0.48)	15/15	28.52 (0.99)	15/15
		VTM	6	UND	0/6	35.70 (0.97)	6/6	22.93 (1.32)	5/6

Table 14 Summary of contrived clinical study results generated on the QuantStudio 7 Flex Real-Time PCR Instrument *(continued)*

Conc.	Total replicates	Transport media	# of samples	MPXV		OPXV		RNaseP	
				Mean C_t (SD[1])	Hit rate	Mean C_t (SD)	Hit rate	Mean C_t (SD)	Hit rate
3X LoD	5	VTM	5	UND	0/5	34.35 (0.45)	5/5	24.82 (2.87)	5/5
5X LoD	5	VTM	5	UND	0/5	32.98 (0.73)	5/5	22.56 (3.29)	5/5

[1] SD: Standard Deviation
[2] One (1) negative specimen in VTM lacked a valid RNase P result and was replaced by an additional negative specimen in VTM.
[3] UND: Undetermined (Negative)

A C_t cutoff values for assay targets

The Pathogen Interpretive Software uses the following C_t cutoff values for assay targets during interpretation of the results.

Table 15 C_t cutoff values

Sample or Control	Target	C_t cutoff		
		QuantStudio™ 5 Real-Time PCR Instrument	QuantStudio™ 5 Dx Real-Time PCR Instrument	QuantStudio™ 7 Flex Real-Time PCR Instrument
Positive control	RNase P	Valid C_t values are ≤36.5		
	Viral targets	Valid C_t values are ≤37		Valid C_t values are ≤38
Negative control	RNase P	Valid C_t values are >36.5		
	Viral targets	Valid C_t values are >37		Valid C_t values are >38
Clinical samples	RNase P	Valid C_t values are ≤35[1]		
	Viral targets	Positive C_t values are ≤37		Positive C_t values are ≤38

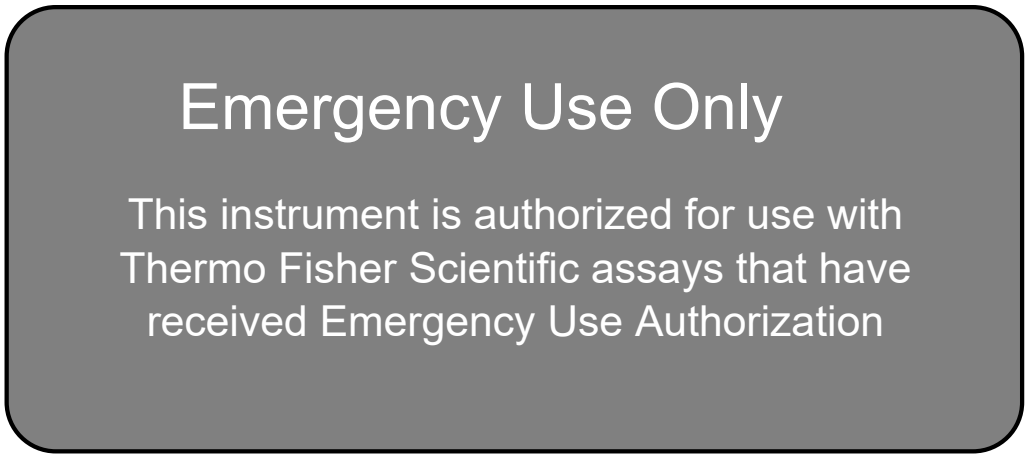
[1] If any of the viral targets are positive, the C_t for RNase P can be >35.

B EUO label for RUO instruments

Affix the Emergency Use Only (EUO) label on each of the following instruments. Retain this labeling throughout the Emergency Use Authorization (EUA) use of the instruments.

- QuantStudio™ 5 Real-Time PCR Instrument
- QuantStudio™ 7 Flex Real-Time PCR Instrument

1. Download or print the following EUO label:



2. Visibly affix the EUO instrument verification label on your instrument. If the instrument includes labeling indicating “For Research Use Only”, cover with the EUO instrument verification label.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication Number
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ 5 Dx IVD Software User Guide	100091229
QuantStudio™ 5 Dx Real-Time PCR Instrument Maintenance and Administration User Guide	100091230
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.3) Maintenance and Administration Guide	4489821
QuantStudio™ Real-Time PCR Software Getting Started Guide	4489822
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit Instructions For Use	MAN0019746
Thermo Scientific™ KingFisher™ Flex User Manual	MAN0019870
Pathogen Interpretive Software Installation Quick Reference	MAN0019535
SAE Administrator Console Dx User Guide[1]	MAN0019574

[1] For use with the Pathogen Interpretive Software.

Customer and technical support

Visit: **http://thermofisher.com/monkeypox** for additional documentation and information about this kit.

For download instructions for the software package, see “Obtain the software package” on page 38.

Visit: **https://www.thermofisher.com/contactus** for service and support information for this kit, including the following:

- Worldwide contact telephone numbers
- Product support information
- Order and web support

- Product documentation such as:
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

TaqPath™ Monkeypox/Orthopox Virus DNA Kit

Multiplex real-time PCR test intended for the qualitative detection of DNA from Monkeypox/Orthopox virus and RNase P internal control in a single reaction well

Catalog Numbers A56978

Pub. No. A57385 Rev. A

For Emergency Use Authorization Only

Intended Use

The Applied Biosystems™ TaqPath™ Monkeypox/Orthopox Virus DNA Kit is a multiplexed polymerase chain reaction (PCR) test intended for the qualitative detection of DNA from monkeypox virus (clade I/II) and non-variola *Orthopoxvirus* in human lesion swab specimens (i.e., swabs of acute pustular and vesicular rash) from individuals suspected of mpox by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of monkeypox virus (clade I/II) and non-variola *Orthopoxvirus* DNA which is generally detectable in human pustular or vesicular lesion specimens during the acute phase of infection. Positive results are indicative of the presence of monkeypox virus (clade I/II) DNA and/or other non-vriola *Orthopoxvirus* DNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results obtained with this device do not preclude monkeypox virus (clade I/II) and/or non-variola *Orthopoxvirus* infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Laboratories within the United States and its territories are required to report test results to the appropriate public health authorities.

The Applied Biosystems™ TaqPath™ Monkeypox/Orthopox Virus DNA Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The Applied Biosystems™ TaqPath™ Monkeypox/Orthopox Virus DNA Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Instructions for Use/Interpretation/Limitations

The complete *TaqPath™ Monkeypox/Orthopox Virus DNA Kit Instructions for Use* (Pub. No. MAN0028390) can be downloaded from the following link: **thermofisher.com/monkeypox**

If you require a printed copy (no charge) or cannot access the document at this location, visit **thermofisher.com/askaquestion** or call 1 800 955 6288 (U.S. only).

This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; use by laboratories certified under CLIA to perform high complexity tests.

This product has been authorized only for the detection of DNA from monkeypox virus and other non-variola orthopoxviruses, not for any other viruses or pathogens.

The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of monkeypox virus, including *in vitro* diagnostics that detect and/or diagnose infection with non-variola *Orthopoxvirus* under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. §360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

IVD

Obtain the software package

To perform data analysis and results interpretation, you must use the following software:

- Applied Biosystems™ Pathogen Interpretive Software v1.1
- SAE Administrator Console Dx v1.0 or SAE Administrator Console Dx v1.2 (for security and audit functions)
- Pathogen Interpretive Software 1.0.0 DAT (SAE Profile)
- Assay panel for your instrument as per *TaqPath™ Monkeypox/Orthopox Virus DNA Kit Instructions for Use*.

To obtain the software, contact your local technical support team. Go to **https://www.thermofisher.com/contactus**, then select **Instrument Service**.

Contents and storage

Table 1 TaqPath™ Monkeypox/Orthopox Virus DNA Kit, 200 reactions (Cat. No. A56978)

Component	Quantity	Amount per tube or bottle	Storage
TaqPath™ Monkeypox/Orthopox Virus Multiplex Assay	1 tube	250 µL	–30°C to –10°C[1]
TaqPath™ Monkeypox/Orthopox Virus DNA Positive Control	1 tube	25 µL	–30°C to –10°C[1]

[1] Do not freeze-thaw more than 5 times.

Customer and technical support

For additional documentation and information about this kit, visit: **thermofisher.com/monkeypox**

For download instructions for the software package, see “Obtain the software package” on page 2. Refer to the Software Release Notes provided with the software before contacting support for the software.

Visit: **https://www.thermofisher.com/contactus** for service and support information for this kit, including worldwide contact telephone numbers, product support information, order and web support, Certificates of Analysis, and Safety Data Sheets (SDSs; also known as MSDSs).

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, California 94566 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. A57385

Revision	Date	Description
A	14 December 2022	New *TaqPath™ Monkeypox/Orthopox Virus DNA Kit Product Information Sheet*.

The customer is responsible for validation of assays and compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

The information in this guide is subject to change without notice.