TaqMan™ Monkeypox Virus Microbe Detection Assay

USER GUIDE

Real-time PCR assay intended for the qualitative detection of nucleic acids from Monkeypox virus

Publication Number MAN0028079
Revision A.0
Revision history:

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.0</td>
<td>15 July 2022</td>
<td>New user guide for the TaqMan™ Monkeypox Virus Microbe Detection Assay.</td>
</tr>
</tbody>
</table>

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

**DISCLAIMER:** TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

**Important Licensing Information:** This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

**Trademarks:** All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a trademark of Roche Molecular Systems, Inc., used under permission and license.

©2022 Thermo Fisher Scientific Inc. All rights reserved.
Contents

■ CHAPTER 1  Product information .................................................. 4
  Product description ........................................................................ 4
  Contents and storage ...................................................................... 4
  Required materials not supplied .................................................. 4

■ CHAPTER 2  Procedural guidelines .............................................. 6
  Guidelines for nucleic acid isolation ........................................... 6
  Guidelines for real-time PCR ...................................................... 6

■ CHAPTER 3  Perform real-time PCR ............................................ 7
  Prepare the real-time PCR reaction plate ........................................ 7
  Set up and run the real-time PCR instrument ................................... 8

■ CHAPTER 4  Analyze the results .................................................. 9

■ APPENDIX A  Reactivity (Inclusivity) .......................................... 10

■ APPENDIX B  Safety ................................................................. 11
  Chemical safety .............................................................................. 12
  Biological hazard safety ................................................................. 13

■ APPENDIX C  Documentation and support .................................... 14
  Related documentation ................................................................ 14
  Customer and technical support .................................................. 14
  Limited product warranty ............................................................. 15
Product description

The Applied Biosystems™ TaqMan™ Monkeypox Virus Microbe Detection Assay is a real-time polymerase chain reaction (PCR) assay designed for the qualitative detection of Monkeypox virus from West African and Congo-Basin clades. The assay can be used with dry swab and swab in viral transport media (VTM) sample types. Monkeypox synthetic DNA positive control can be purchased separately (see “Required materials not supplied” on page 4).

Contents and storage

<table>
<thead>
<tr>
<th>Contents</th>
<th>Cat. No.</th>
<th>Assay ID</th>
<th>Amount</th>
<th>Concentration</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqMan™ Monkeypox Virus Microbe Detection Assay</td>
<td>A50137</td>
<td>Vi07922155_s1</td>
<td>250 µL</td>
<td>20X</td>
<td>–25°C to –15°C</td>
</tr>
</tbody>
</table>

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.
### Real-time PCR

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuantStudio™ 5 Real-Time PCR System, 96-well, 0.2-mL</td>
<td>A28574</td>
</tr>
<tr>
<td>AcroMetrix™ Monkeypox Virus DNA Positive Control</td>
<td>902050 (US region only)</td>
</tr>
<tr>
<td>TaqMan™ Custom DNA Control (1×10^5 copies/µL; 1,000 µL)</td>
<td>A50319</td>
</tr>
<tr>
<td>TaqPath™ BactoPure™ Microbial Detection Master Mix (No ROX™)</td>
<td>A52704</td>
</tr>
<tr>
<td>MicroAmp™ Optical 96-Well Reaction Plate with Barcode</td>
<td>4306737</td>
</tr>
<tr>
<td>TE Buffer</td>
<td>12090015</td>
</tr>
<tr>
<td>Nuclease-Free Water (not DEPC-Treated)</td>
<td>AM9937</td>
</tr>
</tbody>
</table>

### Tubes, plates, and other consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MicroAmp™ Clear Adhesive Film</td>
<td>4306311</td>
</tr>
<tr>
<td>MicroAmp™ Optical Adhesive Film</td>
<td>4311971, 4360954</td>
</tr>
<tr>
<td>MicroAmp™ Adhesive Film Applicator</td>
<td>4333183</td>
</tr>
<tr>
<td>MicroAmp™ Optical Film Compression Pad[^2]</td>
<td>4312639</td>
</tr>
<tr>
<td>Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)</td>
<td>thermofisher.com/plastics</td>
</tr>
<tr>
<td>Sterile aerosol barrier (filtered) pipette tips</td>
<td>thermofisher.com/pipettetips</td>
</tr>
</tbody>
</table>

### Equipment

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory freezers, –30°C to –10°C</td>
<td>MLS</td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Laboratory mixer, vortex or equivalent</td>
<td>MLS</td>
</tr>
<tr>
<td>Single and multichannel adjustable pipettors (1.0 µL to 1.0 mL)</td>
<td>MLS</td>
</tr>
<tr>
<td>Cold block (96-well) or ice</td>
<td>MLS</td>
</tr>
</tbody>
</table>

[^1]: If RNA and DNA analyses are performed on the same plate using a common thermal cycling protocol, the TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (Cat. No. A28522) can be used instead of TaqPath™ BactoPure™ Microbial Detection Master Mix (No ROX™).

[^2]: Required for use with QuantStudio™ 5 series real-time PCR instruments.
Guidelines for nucleic acid isolation

- For detailed instructions on nucleic acid isolation using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with the KingFisher™Flex Purification System, see the appropriate resources that are listed in “Related documentation” on page 14.
- A 200-μL or 400-μL sample input yields approximately 50 μL of purified sample. Depending on the number of assays to be tested, multiple sample aliquots may be needed.

Guidelines for real-time PCR

- Use purified, nondegraded total nucleic acid that is dissolved in PCR-compatible buffer.
- Ensure that the input nucleic acid is free of nuclease or DNase activity and PCR inhibitors.
- To maximize sensitivity, prepare each reaction so that approximately half of the reaction volume is purified total nucleic acid.
- Protect the assays from light and store as indicated until ready for use. Excessive exposure to light can negatively affect the fluorescent probes of the assays.
- *(Optional)* Run technical replicates in triplicate to identify outliers.
Perform real-time PCR

- Prepare the real-time PCR reaction plate .......................................................... 7
- Set up and run the real-time PCR instrument ..................................................... 8

Prepare the real-time PCR reaction plate

**IMPORTANT!** For optimal results, prepare the reaction plate on ice.

Thaw the extracted samples on ice. Resuspend the extracted samples by inverting the tube, then gently vortexing.

1. Gently mix the bottle of TaqPath™ BactoPure™ Microbial Detection Master Mix (No ROX™).

2. To prepare the PCR reaction mix, combine the following components for each reaction, multiplied by the number of required reactions, plus 10% overage.

   **Note:** The following PCR reaction mix volume assumes a sample volume of 9 µL. If you are using <9 µL of sample, increase the volume of reaction mix accordingly by adding Nuclease-Free Water (not DEPC-Treated).

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per well (96-well, 0.2-mL plates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqPath™ BactoPure™ Microbial Detection Master Mix (No ROX™)</td>
<td>10 µL</td>
</tr>
<tr>
<td>TaqMan™ Monkeypox Virus Microbe Detection Assay (20X)</td>
<td>1 µL</td>
</tr>
<tr>
<td>Total PCR reaction mix volume</td>
<td>11 µL</td>
</tr>
</tbody>
</table>

3. Vortex the PCR reaction mix, then centrifuge briefly.

4. Combine the following components in each well of a MicroAmp™ Optical 96-Well Reaction Plate with Barcode.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per well (96-well, 0.2-mL plates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR reaction mix (from step 2)</td>
<td>11 µL[1]</td>
</tr>
<tr>
<td>Extracted nucleic acid sample or elution buffer for NTC[2]</td>
<td>9 µL[3]</td>
</tr>
<tr>
<td>Total volume</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

[1] If using a smaller volume of the extracted nucleic acid sample, adjust the volume of the PCR reaction mix with Nuclease-Free Water (not DEPC-Treated) in step 2, so that the combined volume of the PCR reaction mix and sample is 20 µL per well.

[2] NTC=no template control

[3] The recommended nucleic acid sample volume range is 5 µL–9 µL.
5. Seal the plate with a MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.

**IMPORTANT!** 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.

6. Centrifuge the plate briefly to collect the contents at the bottom of the wells.

**IMPORTANT!** Vortexing and centrifuging are required for proper mixing of the reaction components.

---

### Set up and run the real-time PCR instrument

For detailed instructions to program the thermal cycling conditions or to run the plate on the QuantStudio™ 5 Real-Time PCR System, see “Related documentation” on page 14.

**Note:** The instrument must be configured with a block appropriate for a 96-well, 0.2-mL reaction plate.

1. Select the Fast cycling mode.

   **Note:** The cycling mode depends on the master mix that is used in the reaction. The cycling mode does not depend on the plate format.

2. Set up the thermal protocol for your instrument.

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hold</td>
<td>60°C</td>
<td>30 seconds</td>
<td>1</td>
</tr>
<tr>
<td>Enzyme activation</td>
<td>95°C</td>
<td>2 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95°C</td>
<td>10 seconds</td>
<td>40</td>
</tr>
<tr>
<td>Anneal / Extend</td>
<td>60°C</td>
<td>30 seconds</td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>60°C</td>
<td>30 seconds</td>
<td>1</td>
</tr>
</tbody>
</table>

3. Set the reaction volume to **20 µL**.

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

5. 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.

6. Start the run.
Analyze the results

**IMPORTANT!** It is the responsibility of the laboratories using the TaqMan™ Monkeypox Virus Microbe Detection Assay to design and validate their own experimental design and analysis parameters.

*(Recommended)* Use QuantStudio™ Design and Analysis Software v2.5 or later for data analysis. For more information about using the software, see “Related documentation” on page 14.

**Note:** QuantStudio™ Design and Analysis Desktop Software reports C_q values instead C_t values. The C_q values are equivalent to the C_t values indicated for data analysis and interpretation.

1. In the QuantStudio™ Design and Analysis Desktop Software home screen, open the data file (EDS).
2. In the open data file, click **Actions > Save As**, then save the data file with a new name.
3. For Positive Control samples, change the sample **Type** from **Standard** to **Positive Control**.
4. In the analysis settings, select automatic baseline with a start cycle of **5** and an end cycle of **auto**.
5. Set the appropriate threshold values for each target, as validated by your laboratory.
   **IMPORTANT!** Do not use automatic threshold values.
6. Determine C_t/C_q cutoff values for each target for samples and controls.
7. Analyze results according to analysis, interpretation, and QC parameters, as validated by your laboratory.

For more information, contact Support.
Reactivity (Inclusivity)

*In silico* analysis was executed using 395 sequences from the GISAID database and 247 sequences from NCBI database on July 7, 2022. Only full length or near full length sequences were evaluated. 96% of the sequences showed 100% homology to the assays, while 4% had an insignificant mismatch at the 5’ end of a primer, which is not expected to impact performance.
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.
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.
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.

Documentation and support

- Related documentation ............................................................... 14
- Customer and technical support ........................................................ 14
- Limited product warranty .............................................................. 15

Related documentation

<table>
<thead>
<tr>
<th>Document</th>
<th>Publication number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit User Guide</em></td>
<td>MAN0024756</td>
</tr>
<tr>
<td><em>Procedures for viral nucleic acid isolation User Bulletin</em></td>
<td>MAN0019332</td>
</tr>
<tr>
<td><em>KingFisher™ Flex Purification System User Guide</em></td>
<td>MAN0019870</td>
</tr>
<tr>
<td><em>PureLink™ Viral RNA/DNA Mini Kit User Guide</em></td>
<td>MAN0000562</td>
</tr>
<tr>
<td><em>TaqPath™ BactoPure™ Microbial Detection Master Mix User Guide</em></td>
<td>MAN0025689</td>
</tr>
<tr>
<td><em>TaqPath™ 1-Step Multiplex Master Mix User Guide</em></td>
<td>MAN0014269</td>
</tr>
<tr>
<td><em>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</em></td>
<td>MAN0010407</td>
</tr>
<tr>
<td><em>QuantStudio™ Design and Analysis Software v2 User Guide</em></td>
<td>MAN0018200</td>
</tr>
</tbody>
</table>

Customer and technical support

Visit [thermofisher.com/support](http://thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
• Product documentation
  – User guides, manuals, and protocols
  – 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.