BioGX
Molecular Made Easy

Xfree™ hMPXV Open System PCR Reagents

REF 450-086-E-PXL

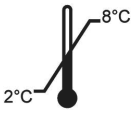
Σ 16 Reactions Extraction-Free Direct Sample Tests

Σ 64 Reactions Extracted Sample Tests

Product Insert

For Research Use Only: Not intended for In Vitro Diagnostic Use

For use with BioGX pixl.16 real-time PCR platform



RUO

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For Research Use Only

Research use only reagents are not intended for human or animal diagnostic use. It is the responsibility of the end user to determine the performance of the reagents in an appropriately designed validation study for their intended use.

The BioGX *Xfree*™ human Monkeypox Virus (hMPXV) real-time PCR-based detection reagent is manufactured and packaged as an open system reagent (OSR) for use with BioGX pixl.16 real-time PCR platform (for Android® based software version 1.6.9 or later).

PLEASE READ ENTIRE PACKAGE INSERT BEFORE PROCEEDING TO USE THE OSR.

PRODUCT OVERVIEW

The BioGX *Xfree*™ Sample-Ready™ OSR has been formulated in lyophilized format for the multiplex real-time PCR-based detection of DNA from human Monkeypox virus (G2R_G gene[1,2]), human Orthopox virus (E9L NVAR gene[3]) and human RNase P gene to serve as an endogenous extraction control.

Note:

OSR for BioGX pixl.16 Platform (450-086-E-PXL) contains all PCR primers, probes, enzymes, dNTPs, $MgCl_2$, buffers, and other components required for the PCR reaction. No exogenous addition of SPC is needed.

PACKAGE CONTENTS

BioGX REF: 450-086-E-PXL
Platform: BioGX pixl.16

Each 16-reaction/64-reaction package consists of two pouches:

1. The first pouch contains 2 x 8-tube PCR strips. Each tube contains BioGX lyophilized Sample-Ready™ reagents sufficient for 16 PCR reactions when used in extraction-free format and for 64 PCR reactions when used in extracted format.

2. The second pouch contains 1 tube of Rehydration Buffer E (BioGX catalog no. 800-0031-E-L), each containing 650µL of Rehydration Buffer E.

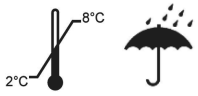
EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- External Positive Control[s] available from BioGX or Microbix
  - BioGX Lyophilized Positive Control Template DNA Beads (10^5 copies/bead)
    - BioGX Monkeypox (Part number 720-0232)
    - BioGX Orthopox (Part number 720-0233)
    - BioGX RNaseP (DNA) (Part number 720-0009)
  - Microbix PROCEEDx™FLOQ® Monkeypox West African Positive Swab (Microbix catalog no. VP-S-81-02)
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent
- Disposable nitrile gloves
- BioGX pixl.16 real-time PCR instrument (BioGX catalog no. 650-016-PXL)
- BioGX pixl.16 consumables.
  - TempAssure® PCR 8-Tube Strips, Att. Optical Caps (BioGX catalog no. 010-280-ETS) or
  - EasyStrip™ Plus Tube Strip with Attached Ultra Clear Caps (Thermo Fisher catalog no. AB2005) or
  - Axygen® 0.2 mL Polypropylene PCR Tube Strips and attached Flat Cap Strips, 8 Tubes/Strip, (Corning catalog no.: PCR-0208-AF-C)
  - Optional: BioGX pixl Barcode Scanner with Stand (BioGX catalog no. 650-726-SC-PXL)
- Calibrated micropipettes

WARNINGS AND PRECAUTIONS

- For research use only. Not intended for human or animal diagnostics use.
- If handling biological samples, including used Extraction Kits and PCR Cartridges, treat as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29[4] and in Biosafety in Microbiological and Biomedical Laboratories[5].
- BioGX REF: 450-086-E-PXL has been quality control tested only with the BioGX pixl.16 real-time PCR platform.
- Do not use the reagents if the protective pouches are open or torn upon arrival.
- Close reagent protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing and store at 2-8 °C.
- Do not remove desiccant from the PCR master mix pouches.
- Do not use Sample-Ready™ master mix if the desiccant is not present or is broken inside the Sample-Ready™ master mix pouches.
- Do not use reagent tubes if the foil seal has been opened or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not use expired reagents and/or materials.
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where samples or kits are being handled.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations.
- Use clean gloves when handling PCR reagents and buffer tubes.

STORAGE REQUIREMENTS AND RECOMMENDATIONS



Reagents are stable at a temperature range of 2-30°C during shipment for 5 days, but BioGX recommends long-term storage at 2-8°C. Reagents have been tested to demonstrate optimal performance when stored properly and consumed by the Manufacturer Recommended Use By Date. The end user may opt to extend the useful life for Research Use Only reagents upon completing their own performance validations. BioGX's guarantee of reagent integrity does not extend beyond the Manufacturer Recommended Use By Date when stored properly. Avoid exposing the reagents (lyophilized or rehydrated) to direct sunlight or long-term ambient lighting. Tightly reseal the pouch with unused reactions and immediately return to a refrigerator after opening. To mitigate reagent performance degradation from exposure to moisture, BioGX suggests using the entire contents of the opened pouch within 1 month; however, the user may choose to verify an extended working time > 1 month by performance testing with positive controls and an examination of the sample preparation control target.

SAMPLE TYPES UTILIZED FOR QUALITY CONTROL TESTING

-Lesion swab samples collected in Copan Universal Transport Media (UTM®), Copan ESwab™, Viral Transport Media (VTM)[a], saline (0.85% NaCl), Phosphate Buffer Saline (PBS)[b]

REAGENT OPTICAL CONFIGURATION

Table 1. Optical Channel Configuration for REF 450-086-E-PXL

Optical Channel (Fluorophore Equivalent)	**Target**
FAM	Monkeypox virus
HEX	Orthopox virus
Texas Red	Unused
Cy5	RNase P
Cy5.5	N/A

[a]Centers for Disease Control. Preparation of Viral Transport Media (SOP#: DSR-052-05)
[b]Phosphate Buffered Saline formulation: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.2-7.4

QUALITY CONTROL AND TESTING PARAMETERS

BioGX pixl.16 Platform

As a starting point, users can import and install a PCR run file onto:

1. BioGX pixl.16 real-time PCR platform (For Android® based software version 1.6.9 or later)

BioGX's most current BioGX pixl.16 PCR run files utilized for quality control of this product can be obtained by sending an email to TS@biogx.com. Please refer to the BioGX pixl.16 user manual[6] for uploading instructions.

PREPARATION OF MICROBIX EXTERNAL CONTROL

Positive Control Microbix PROCEEDx™ FLOQ® Monkeypox West African Positive Swab (Microbix catalog no. VP-S-81-02) is added into Copan Universal Transport Media (UTM®), Copan ESwab™, Viral Transport Media (VTM), saline (0.85% NaCl) or Phosphate Buffer Saline (PBS) and incubated at room temperature for 1-2 min. After the incubation, swirl the swab 5-10 times in the vial, express the swab on the inside of the vial and discard into designated biohazard waste.

Direct Sample Processing of Microbix External Control

Transfer **20 µL** of direct positive control sample to the rehydrated BioGX master mix as described in Section: *Direct Sample - Assembly of BioGX Reagents.*

Extracted Sample Processing of Microbix External Control

Thoroughly vortex the sample prior to processing. Follow manufacturer recommendations for extraction of appropriate sample volume. Transfer 5 µL of purified nucleic acid to master mix as described in Section: *Extracted Sample - Assembly of BioGX Reagents.*

The External Control available from Microbix (Microbix PROCEEDx™ FLOQ® Monkeypox West African Positive Swab (Microbix catalog no. VP-S-81-02)) is treated as if it was a patient sample. Refer to **Table 2** in the "Results Interpretation" section for the interpretation of External Control assay results. It is recommended that one (1) External Positive Control and one (1) External Negative Control be included with each run of patient samples. BioGX recommends that the External Negative Control be prepared prior to the External Positive Control in order to reduce the potential for cross contamination as a result of control preparation.

For further reference, please refer to the product information sheet for Microbix PROCEEDx™ FLOQ® Monkeypox West African Positive Swab (Microbix catalog no. VP-S-81-02)[7].

SAMPLE VOLUMES UTILIZED DURING QUALITY CONTROL TESTING

The end user may choose to validate a different pretreatment method or volume of sample to load other than the sample processing used by BioGX for QC testing as outlined below.

PCR SET-UP FOR DIRECT SAMPLES on BioGX pixl.16 Platform

Swab sample (3 mL Copan UTM®, VTM, saline (0.85% NaCl) or PBS)

BioGX pixl.16 Platform
Thoroughly vortex the sample prior to processing. Transfer **20 µL** of direct sample to the rehydrated BioGX master mix as described in Section: *Direct Sample - Assembly of BioGX Reagents.*

Swab sample (1 mL Copan UTM®, Copan ESwab™, VTM, saline (0.85% NaCl) or PBS)

BioGX pixl.16 Platform
Thoroughly vortex the sample prior to processing. Transfer **20 µL** of direct sample to the rehydrated BioGX master mix as described in Section: *Direct Sample - Assembly of BioGX Reagents.*

PCR SET-UP FOR EXTRACTED SAMPLES on BioGX pixl.16 Platform

Swab sample (3 mL Copan UTM®, VTM, saline (0.85% NaCl) or PBS)

BioGX pixl.16 Platform

Thoroughly vortex the sample prior to processing. Follow manufacturer recommendations for extraction of appropriate sample volume. Transfer 5 µL of purified nucleic acid to master mix as described in Section: *Extracted Sample - Assembly of BioGX Reagents.*

Swab sample (1 mL Copan UTM®, Copan ESwab™ , VTM, saline (0.85% NaCl) or PBS)

BioGX pixl.16 Platform

Thoroughly vortex the sample prior to processing. Follow manufacturer recommendations for extraction of appropriate sample volume. Transfer 5 µL of purified nucleic acid to master mix as described in Section: *Extracted Sample - Assembly of BioGX Reagents.*

WEAR NITRILE GLOVES WHEN HANDLING LYOPHILIZED REAGENTS TO REDUCE THE GENERATION OF STATIC CHARGES. DO NOT USE LATEX GLOVES.

Direct Sample - Assembly of BioGX Reagents

1. Prepare the appropriate number of PCR tube strips.
2. Transfer **30 µL** of Rehydration Buffer E to one vial of lyophilized BioGX reagents. The rehydrated master mix is sufficient for 1 sample to be tested.

Note: 8-tube strips containing reagents are only compatible with the BioGX pixl.16 platform.

3. Mix by gently tapping the tube with your fingers until the lyophilized pellet has been dissolved.

(IMPORTANT: Keep rehydrated master mix in a cold block or on ice if setup cannot be completed within 20 minutes. If the rehydrated master mix cannot be used immediately, it can be capped and stored up to 24 hours at 2-8°C, protected from light).

4. To each PCR tube containing **30 µL** of rehydrated master mix, add **20 µL** of direct sample.
5. Affix the optical caps to the PCR tube strips.
6. Pulse spin the sealed PCR tube strips.
7. Load PCR tube strips into the real-time PCR platform and start the run. Avoid unnecessary delay once tubes are loaded into the real-time PCR instrument.

PCR SET-UP FOR EXTRACTED SAMPLES on BioGX pixl.16 Platform

Extracted Sample - Assembly of BioGX Reagents

1. Prepare the appropriate number of 8-tube PCR strips
2. Transfer **40 µL** of molecular grade water to one vial of lyophilized BioGX reagents. The rehydrated master mix is more than sufficient for 4 samples to be tested.

Rehydrated master mix must be transferred to clean pixl-compatible 8-tube PCR strips.

3. Mix by gently pipetting up and down.

(IMPORTANT: Keep rehydrated master mix in a cold block or on ice if setup cannot be completed within 20 minutes. If the rehydrated master mix cannot be used immediately, it can be capped and stored up to 24 hours at 2-8°C, protected from light).

4. Transfer **10 µL** of rehydrated master mix to the bottom of **4 empty tubes**.
5. To each well containing **10 µL** of rehydrated master mix, add **5 µL** of extracted sample.
5. Affix the optical caps to the PCR tube strips.
6. Pulse spin the sealed PCR tube strips.
7. Load PCR tube strips into the real-time PCR platform and start the run. Avoid unnecessary delay once tubes are loaded into the real-time PCR instrument.

ASSAY PERFORMANCE

All BioGX Research Use Only products are designed to detect 20 copies or less of the target nucleic acid per reaction.

INTERPRETATION OF RESULTS

Table 2. Interpretation of Microbix External Control for 450-086-E-PXL.

Control Type	Applicability for Monitoring	Monkeypox virus	Orthopox virus	RNase P
Microbix PROCEEDx™ FLOQ® Monkeypox West African Positive Swab (Microbix catalog no. VP-S-81-02)	Substantial reagent failure including primer and probe integrity	+	+	+

Note: Microbix PROCEEDx™ FLOQ® Monkeypox West African Positive Swab (Microbix catalog no. VP-S-81-02) contains human cellular material to control for the amplification of human RNase P.

Table 3. Multiplex PCR Results Interpretation for 450-086-E-PXL.

Monkeypox virus	Orthopox virus	RNase P	Interpretation
+	+	+/-	Monkeypox virus POSITIVE
-	+/-	+	Monkeypox virus NEGATIVE
-	+	+/-	Orthopox virus POSITIVE*
-	-	+	Monkeypox and Orthopox virus NEGATIVE
-	-	-	Unresolved**

*Orthopox only amplification should be retested to confirm lack of detection of Monkeypox detection was due to poor collection. Upon retesting, Orthopox only detection indicates detection of a non-Monkeypox virus.
**Failed PCR due to inhibition, reagent failure or incorrect assembly of PCR reaction.

REFERENCES

1. Li, Daniel, et al. "Evaluation of the GeneXpert for human monkeypox diagnosis." The American Journal of Tropical Medicine and Hygiene 96.2 (2017): 405.
2. Li, Yu, et al. "Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA." Journal of virological methods 169.1 (2010): 223-227.
3. Li, Yu, et al. "Detection of monkeypox virus with real-time PCR assays." Journal of Clinical Virology 36.3 (2006): 194-203.
4. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
5. Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. Choosewood L.C. and Wilson D.E. (eds) (2009). HHS Publication No. (CDC) 21-1112.
6. pixl™ Real-Time PCR Platform Instructions for Use Manual (refer to the latest version)
7. Microbix, PROCEEDx™FLOQ® Monkeypox West African Swab Positive Sample VP-S-81-02, Product Information Sheet (Refer to the latest edition).

Please call BioGX or email info@biogx.com with any questions you may have regarding this product.

Rev. #	Effective Date	Summary of Changes
04	22 AUG 2023	Addition of Microbix PROCEEDx™FLOQ® Monkeypox West African Positive Swab (Microbix catalog no. VP-S-81-02) as positive control alternative
03	20 JAN 2023	Update of title page to include number of tests per kit for extraction-free and extracted workflow
02	06 JAN 2023	Addition of extraction based workflow
01	01 SEP 2022	Initial Release.

SYMBOLS

Symbol	Meaning	Symbol	Meaning
	Catalog number		Contains sufficient for <n> tests
	Research Use Only		Manufacturer
	Keep dry		Temperature limitation
	Biological Risks		

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