



BioGX
Molecular Made Easy

Xfree[™] COVID-19, Flu A, Flu B Open System PCR Reagents

REF 450-090-H-PXL



16 Extraction-Free Direct Sample Tests

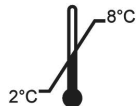


64 Extracted Sample Tests

Product Insert

For Research Use Only: Not for use in diagnostic procedures

For use with ABI QuantStudio[™] 5, Bio-Rad CFX96 Touch[™], BioGX pixl.16
real-time PCR platform



RUO

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For Research Use Only

Research use only reagents are not for use in diagnostic procedures. It is the responsibility of the end user to implement for the intended use.

The Xfree COVID-19, Flu A, Flu B real-time PCR-based detection reagent is manufactured and packaged as an open system reagent (OSR) for use with open system platforms and has to be validated by the user. Examples of open system platforms are the Applied Biosystems QuantStudio™ 5 (Design & Analysis software version 1.5.1 or later), Bio-Rad CFX96 Touch™, or BioGX pixl.16 (For Android® based software version 1.6.9 or later) real-time PCR platforms.

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

PRODUCT OVERVIEW

The BioGX Sample-Ready™ OSR has been formulated in lyophilized format for the multiplex real-time PCR-based detection of RNA from SARS-CoV-2 (RNA-dependent RNA polymerase gene (RdRp genes^{1,2}) and Nucleocapsid phosphoprotein gene region N1³⁻⁶), Influenza A (matrix gene⁷), Influenza B (nonstructural gene⁷) and single stranded RNA (ssRNA) to serve as an Internal Amplification Control (IAC).

Note:

OSR 450-090-H-PXL contains all PCR primers, probes, enzymes, dNTPs, MgCl₂, buffers, and other components required for the PCR reaction. No exogenous addition of IAC is needed.

PACKAGE CONTENTS

BioGX REF: 450-090-H-PXL

Platform(s): ABI QuantStudio™ 5, Bio-Rad CFX96 Touch™, 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 1 PCR reaction. Each pouch contains reagents 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 H (BioGX catalog no. 800-0034-HL), each containing 750 µL of Rehydration Buffer H.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- External Positive Control[s] available from BioGX or Microbix
 - Lyophilized Positive Control Template DNA Beads (10^5 copies/bead)
 - BioGX SARS CoV-2 Synthetic Nucleocapsid Phosphoprotein Gene Region (N1) (BioGX catalog no. 720-0206)
 - BioGX SARS-CoV-2 RNA-Dependent RNA Polymerase (RdRp) (BioGX catalog no. 720-0209).
 - BioGX Influenza A (BioGX catalog no. 720-0002)
 - BioGX Influenza B (BioGX catalog no. 720-0003)
 - Microbix PROCEEDx™ FLOQ® SARS-CoV-2 Positive Swab (Microbix catalog no. VP-S-19-01)
 - Microbix PROCEEDx™ FLOQ® Influenza A Positive Swab (Microbix catalog no. VP-S-13-M1)
 - Microbix PROCEEDx™ FLOQ® Influenza B Positive Swab (Microbix catalog no. VP-S-14-M1)
- Disposable nitrile gloves
- Applied Biosystems QuantStudio 5 (0.2 mL) consumables.
 - Thermo Fisher optical 8-tube strip (catalog no. 4316567)
 - Thermo Fisher ultra-clear optical caps, strips of 8 (catalog no. AB-0866)
 - Thermo Fisher 96-well optical clear reaction plates (catalog no. A36924)
 - Thermo Fisher 96-well qPCR plate seals (catalog no. AB-1170)
- Bio-Rad CFX96 Touch consumables.
 - Bio-Rad 8-tube PCR strips without caps (catalog no. TLS0851)
 - Bio-Rad Optical flat 8-cap strips for PCR tubes (catalog no. TCS0803)
 - Bio-Rad 96-well plates (catalog no. HSP9655)
 - Bio-Rad 96-well plate sealing film, optical (catalog no. MSB1001)
- BioGX pixl™ Real-Time PCR Platform (pixl.16™) (BioGX catalog no. 650-016-PXL)
- BioGX pixl™ Real-Time PCR Platform (pixl.16™) instrument consumables (0.2 mL)
 - TempAssure® PCR 8-Tube Strips, Att. Optical Caps (BioGX catalog no. 010-280-ETS)
 - 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)
- Calibrated micropipettes

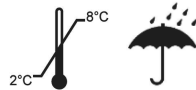
- **Optional:**
 - BioGX pixl Barcode Scanner with Stand (BioGX catalog no. 650-726-SC-PXL)

WARNINGS AND PRECAUTIONS



- For research use only. Not intended for use in diagnostic procedures.
- 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 M29⁸ and in Biosafety in Microbiological and Biomedical Laboratories⁹.
- BioGX REF: 450-090-H-PXL has been quality control tested only with the ABI QuantStudio™ 5, Bio-Rad CFX96 Touch™ and BioGX pixl.16 real-time PCR platforms.
- 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

-Nasal 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 450-090-H-PXL.

Optical Channel (Fluorophore Equivalent)	Target
FAM	SARS-CoV-2 (RdRp and N1 gene)
HEX	Influenza B
Texas Red	Influenza A
Cy5	Internal Amplification Control (ssRNA)
Cy5.5	Unused

^aCenters for Disease Control. Preparation of Viral Transport Media (SOP#: DSR-052-05)

^bPhosphate Buffered Saline formulation: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.2-7.4

QUALITY CONTROL AND TESTING PARAMETERS

ABI, Bio-Rad and BioGX pixl.16 Platforms

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

1. Applied Biosystems QuantStudio™ 5 (Design & Analysis software version 1.5.1 or later)
2. Bio-Rad CFX96 Touch™ (Maestro software version 1.1 or later)
3. BioGX pixl.16 real-time PCR platform (For Android® based software version 1.6.9 or later)

BioGX's most current ABI QuantStudio™ 5, Bio-Rad CFX96 Touch™ and 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 Applied Biosystems QuantStudio™ 5 user manual¹⁰ for uploading instructions. Please refer to the Bio-Rad CFX96 Touch™ user manual¹¹ for uploading instructions. Please refer to the BioGX pixl.16 user manual¹² for uploading instructions.

PREPARATION OF MICROBIX EXTERNAL CONTROL

Positive Control Microbix PROCEEDx™FLOQ® SARS-CoV-2 Positive Swab, (Microbix catalog no. VP-S-19-01), Microbix PROCEEDx™FLOQ® Influenza A Positive Swab (Microbix catalog no. VP-S-13-M1), Microbix PROCEEDx™FLOQ® Influenza B Positive Swab (Microbix catalog no. VP-S-14-M1), is added individually 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 - UTM®, VTM, saline or PBS

Transfer **20 µL** of direct positive control sample in UTM®, VTM, saline or PBS buffer to the BioGX master mix rehydrated with **30 µL** Buffer H as described in Section: *Direct Sample - Assembly of BioGX Reagents*.

Direct Sample Processing of Microbix External Control - ESwab™

Transfer **5 µL** of direct positive control sample in ESwab™ buffer to the BioGX master mix rehydrated with **45 µL** Buffer H 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 Controls available from Microbix (Microbix PROCEEDx™FLOQ® SARS-CoV-2 Positive Swab (Microbix catalog no. VP-S-19-01), Microbix PROCEEDx™FLOQ® Influenza A Positive Swab (Microbix catalog no. VP-S-13-M1), Microbix PROCEEDx™FLOQ®

Influenza B Positive Swab (Microbix catalog no. VP-S-14-M1) are treated as if they were 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 reference the product information sheets for Microbix PROCEEDx™FLOQ® SARS-CoV-2 Positive Swab (Microbix catalog no. VP-S-19-01)¹³, Microbix PROCEEDx™FLOQ® Influenza A Positive Swab (Microbix catalog no. VP-S-13-M1)¹⁴, Microbix PROCEEDx™FLOQ® Influenza B Positive Swab (Microbix catalog no. VP-S-14-M1)¹⁵.

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

Swab sample (1mL or 3 mL Copan UTM[®], VTM, saline (0.85% NaCl) or PBS)

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 ESwab™)

Thoroughly vortex the sample prior to processing. Transfer **5 µL** of direct sample to the rehydrated BioGX master mix as described in Section: *Direct 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 - UTM[®], VTM, saline (0.85% NaCl) or PBS

1. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
2. Transfer **30 µL** of Rehydration Buffer H to one tube 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. 8-tube strips containing reagents ARE NOT compatible with Applied Biosystems or Bio-Rad Platforms. Rehydrated master mix must be transferred to 8-tube PCR tube strips or 96-well PCR plates compatible with appropriate instruments.

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/seals to the PCR tube strips/plates.
6. Pulse spin the sealed PCR tube strips/plates.
- 7a. **For BioGX pixl.16 Platform:** Load PCR tube strips into the real-time PCR platform and start the run. Avoid unnecessary delay once samples are loaded into the real-time PCR instrument.
- 7b. **For Applied Biosystems and Bio-Rad Platforms:** Transfer rehydrated master mix to 8-tube PCR tube strips or 96-well PCR plates compatible with the appropriate instrument and start the run. Avoid unnecessary delay once samples are loaded into the real-time PCR instrument.

Direct Sample - Assembly of BioGX Reagents - ESwab™

1. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
2. Transfer **45 µL** of Rehydration Buffer H to one tube 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. 8-tube strips containing reagents ARE NOT compatible with Applied Biosystems or Bio-Rad Platforms. Rehydrated master mix must be transferred to 8-tube PCR tube strips or 96-well PCR plates compatible with appropriate instruments.

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 **45 µL** of rehydrated master mix, add **5 µL** of direct **ESwab™** sample.

5. Affix the optical caps/seals to the PCR tube strips/plates.

6. Pulse spin the sealed PCR tube strips/plates.

7a. **For BioGX pixl.16 Platform:** Load PCR tube strips into the real-time PCR platform and start the run. Avoid unnecessary delay once samples are loaded into the real-time PCR instrument.

7b. **For Applied Biosystems and Bio-Rad Platforms:** Transfer rehydrated master mix to 8-tube PCR tube strips or 96-well PCR plates compatible with the appropriate instrument and start the run. Avoid unnecessary delay once samples are loaded into the real-time PCR instrument.

PCR SET-UP FOR EXTRACTED SAMPLES

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

Validated magnetic bead or silica column extraction kits

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)

Validated magnetic bead or silica column extraction kits

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.

Extracted Sample - Assembly of BioGX Reagents

1. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
2. Transfer **40 µL** of Buffer H to one tube of lyophilized BioGX reagents. The rehydrated master mix is more than sufficient for 4 samples to be tested.

Note: Rehydrated master mix must be transferred to clean 8-tube PCR strips or 96-well PCR plates compatible with appropriate Applied Biosystems, Bio-Rad and BioGX pixl.16 platform.

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 clean, 8-tube PCR strips or 96-well PCR plates compatible with appropriate Applied Biosystems, Bio-Rad and BioGX pixl.16 platform.**
5. To each tube containing **10 µL** of rehydrated master mix, add **5 µL** of extracted sample.
5. Affix the optical caps/seals to the PCR tube strips/plates.
6. Pulse spin the sealed PCR tube strips/plates.
7. Load PCR tube strips/plates into the real-time PCR platform and start the run. Avoid unnecessary delay once samples are loaded into the real-time PCR instrument.

ASSAY PERFORMANCE

All BioGX Research Use Only products are designed to detect 50 copies or less of the target nucleic acid per reaction.

INTERPRETATION OF RESULTS

Table 2. Interpretation of Microbix external controls for 450-090-H-PXL.

Control Type	Applicability for Monitoring	SARS-CoV-2	Influenza A	Influenza B	IAC
Microbix PROCEEDx™FLOQ® SARS-CoV-2 Positive Swab (Microbix catalog no. VP-S-19-01)	Substantial reagent failure including primer and probe integrity	+	-	-	+
Microbix PROCEEDx™FLOQ® Influenza A Positive Swab (Microbix catalog no. VP-S-13-M1)	Substantial reagent failure including primer and probe integrity	-	+	-	+
Microbix PROCEEDx™FLOQ® Influenza B Positive Swab (Microbix catalog no. VP-S-14-M1)	Substantial reagent failure including primer and probe integrity	-	-	+	+

Note: Microbix PROCEEDx™FLOQ® SARS-CoV-2 Positive Swab (Microbix catalog no. VP-S-19-01), Microbix PROCEEDx™FLOQ® Influenza A Positive Swab (Microbix catalog no. VP-S-13-M1), Microbix PROCEEDx™FLOQ® Influenza B Positive Swab (Microbix catalog no. VP-S-14-M1) contain human cellular material.

Table 3. Multiplex PCR Results Interpretation for 450-090-H-PXL.

SARS-CoV-2	Influenza A	Influenza B	IAC	Interpretation
+	-	-	+/-	SARS-CoV-2 POSITIVE
-	+	-	+/-	Influenza A POSITIVE
-	-	+	+/-	Influenza B POSITIVE
-	-	-	+	SARS-CoV-2, Influenza A, Influenza B NEGATIVE
-	-	-	-	Unresolved*

*Failed PCR due to inhibition, reagent failure or incorrect assembly of PCR reaction.








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10. QuantStudio™ Design and Analysis software User Guide, ThermoFisher Scientific, Waltham, Massachusetts, USA (Refer to the latest version).
11. CFX96 Touch, CFX96 Touch Deep Well, CFX Connect, and CFX384 Touch Systems Instruction Manual (refer to the latest version), Bio-Rad Laboratories, Inc., Hercules, California, USA (Refer to the latest version).
12. BioGX pixl.16 Real-Time PCR Platform Instructions for Use (refer to the latest version), BioGX, Inc., Birmingham, Alabama, USA.
13. Microbix, PROCEEDx™FLOQ® SARS-CoV-2 Swab Positive Sample VP-S-19-01, Product Information Sheet (Refer to the latest edition).
14. Microbix, PROCEEDx™FLOQ® Respiratory NAAT Influenza A Swab Positive Sample VP-S-13-M1, Product Information Sheet (Refer to the latest edition).
15. Microbix, PROCEEDx™FLOQ® Respiratory NAAT Influenza B Swab Positive Sample VP-S-14-M1, Product Information Sheet (Refer to the latest edition).

REVISION HISTORY

Rev. #	Effective Date	Summary of Changes
03	10 NOV 2025	Updated research use only text, update Eswab sample volume for direct sample workflow, updated Buffer H tube volume and Buffer H use in rehydration of master mix for extracted workflow.
02	04 MAR 2024	Update to recommend direct sample and extracted sample workflow with BioGX pixl.16, ABI QuantStudio™ 5 and Bio-Rad CFX96™ Touch platform
01	18 AUG 2023	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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