



BioGX

Molecular Made Easy

X_{free} SARS-CoV-2 (N1, RdRp), RNase P, IAC Extraction-Free PCR - Multi-Platform Open System PCR Reagents

REF 450-085-XMP

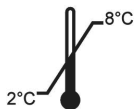


104 Reactions

Product Insert

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

For use with ABI QuantStudio™ 5, ABI 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™, 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.

This product 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), Applied Biosystems 7500 Fast Dx (SDS software version 1.4 or later), Bio-Rad CFX96 Touch™, CFX384 Touch™ (Maestro software version 1.1 or later) 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 virus (N1; nucleocapsid phosphoprotein gene^{1,2,3} and RdRp; RNA-dependent RNA polymerase gene^{4,5}), human RNase P (Endogenous Sample Processing Control (SPC)) and Internal Amplification Control (synthetic single-stranded RNA (IAC)). The SPC serves as an endogenous sample control and the IAC serves as reverse transcription and PCR amplification control. For environmental testing applications^{6, 7, 8}, the Xfree SARS-CoV-2 reagents provide laboratories with flexibility for analysis of various sample types.

One format for the lyophilized Sample-Ready OSR kit is available:

BioGX REF: 450-085-XMP

Platform(s): ABI 7500 Fast Dx, ABI QuantStudio™ 5, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™, BioGX pixl.16

OSR for ABI, Bio-Rad or BioGX pixl.16 Platforms (450-085-XMP) contain all PCR primers, probes, enzymes, dNTPs, MgCl₂, buffers, and other components required for the PCR reaction. Single stranded RNA sequence is included in the OSR master mix and serves as Internal Amplification Control (IAC). No exogenous addition of SPC is needed.

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Each 104-reaction package (direct sample) consists of one pouch containing 4 vials. Each vial contains BioGX lyophilized Sample-Ready™ OSR sufficient for:

- 1.) 40 tests using purified nucleic acid template
- 2.) 26 tests using direct sample addition
- 3.) 13 tests using pooled direct sample addition (5 sample pools)

Molecular grade water is required to rehydrate the lyophilized reagents.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BioGX Lyophilized Positive Control Template RNA Beads (10^5 copies/bead)
 - SARS-CoV-2 Nucleocapsid phosphoprotein gene (N1) (Part number: 720-0206)
 - SARS-CoV-2 RNA-Dependent RNA Polymerase (RdRp) (Part number: 720-0209)
 - RNase P (Part number: 720-0208)
- BioGX Molecular Grade Water or equivalent
 - BioGX Rehydration Water (Part number: 800-0035-12)
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent
- 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)
- Applied Biosystems 7500 Fast Dx (0.1 mL) consumables.
 - Thermo Fisher optical 8-tube strip (catalog no. 4358293)
 - Thermo Fisher ultra-clear optical caps, strips of 8 (catalog no. 4323032)
 - Thermo Fisher 96-well optical reaction plates (catalog no. 4346906)
 - Thermo Fisher 96-well qPCR plate seals (catalog no. 4311971)
- 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)
- Bio-Rad CFX384 Touch consumables.
 - Bio-Rad 384-well plates (catalog no. HSP3905)
 - Bio-Rad 384-well plate sealing film, optical (catalog no. MSB1001)

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- BioGX pixl.16 (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)

WARNINGS AND PRECAUTIONS

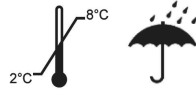


- For research use only. Not intended for human or animal diagnostics use.
- If handling biological samples, treat as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29⁹ and in Biosafety in Microbiological and Biomedical Laboratories¹⁰.
- This test has been optimized only with the Applied Biosystems QuantStudio™ 5, Applied Biosystems 7500 Fast Dx, Bio-Rad CFX96 Touch™, CFX384 Touch™, or 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 master mix if the desiccant is not present or is broken inside the pouches. Do not use reagent vials if they are 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.
- Clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water.
- Use clean gloves when handling PCR reagents.



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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 performance validations. BioGX's guarantee of reagent integrity does not extend beyond the Manufacturer Recommended Use By Date. Avoid exposing the reagents (lyophilized or rehydrated) to direct sunlight or long-term ambient lighting. Store unused rehydrated master mix up to 24 hours at 2-8°C, protected from light. Tightly reseal the pouch with unused vials 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

-Swab samples collected in Copan Universal Transport Media (UTM[®]) or Copan ESwab[™]

REAGENT OPTICAL CONFIGURATION

Table 1. Optical Channel Configuration for REF 450-085-XMP.

Optical Channel (Fluorophore Equivalent)	Target
FAM	SARS-CoV-2 (RdRp gene)
HEX	IAC
Texas Red	SARS-CoV-2 (N1 gene)
Cy5	RNase P
Cy5.5	Unused

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QUALITY CONTROL, REAGENT PREPARATION AND EXTRACTION TESTING PARAMETERS

ABI, Bio-Rad and BioGX pixl.16 Platforms (BioGX REF: 450-085-XMP)

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. ABI 7500 Fast Dx (SDS software version 1.4 or later)
3. Bio-Rad CFX96 Touch™ (Maestro software version 1.1 or later)
4. Bio-Rad CFX384 Touch™ (Maestro software version 1.1 or later)
5. BioGX pixl.16 real-time PCR platform (For Android® based software version 1.6.9 or later)

BioGX's most current ABI QuantStudio™ 5, ABI 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 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 manual¹¹ for uploading instructions. Please refer to the ABI 7500 Fast Dx user manual¹² for uploading instructions. Please refer to the Bio-Rad CFX96 Touch™ user manual¹³ for uploading instructions. Please refer to the Bio-Rad CFX384 Touch™ user manual¹³ for uploading instructions. Please refer to the BioGX pixl.16 user manual¹⁴ for uploading instructions.

**SARS-CoV-2 (N1, RdRp), RNase P, IAC
Extraction-Free PCR - Multi-Platform
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RT-PCR Set-Up Direct Individual Sample

BioGX Product 450-085-XMP is recommended for use with swab samples collected in 1 or 3 mL collection media (i.e. Copan ESwab™, Copan UTM® , UVT, VTM, or saline).

1. Transfer **400 µL** of **molecular grade water** to one vial of lyophilized BioGX Xfree SARS-CoV-2 reagents. Mix by gently pipetting up and down with 1000 µL pipet tip. (IMPORTANT: Keep rehydrated master mix in a cold block or on ice if set-up 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).
2. Transfer **15 µL** of **rehydrated master mix** to the bottom of **26 empty wells** (8-tube PCR strips or 96-well PCR plate).
3. To each well containing 15 µL of rehydrated master mix, add **5 µL** of **individual direct sample**.
4. Affix the appropriate optical caps or optical plate seals.
5. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.

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RT-PCR Set-Up Direct Pooled Sample

BioGX Product 450-085-XMP is recommended for use with pooled swab samples collected in 1 or 3 mL collection media (i.e. Copan ESwab™, Copan UTM® , UVT, VTM, or saline).

1. Transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree SARS-CoV-2 reagents. Mix by gently pipetting up and down with 1000 µL pipet tip. (IMPORTANT: Keep rehydrated master mix in a cold block or on ice if set-up 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).
2. Transfer **30 µL of rehydrated master mix** to the bottom of **13 empty wells** (8-tube PCR strips or 96-well PCR plate).
3. Prepare one pool of 5 patient samples by transferring a minimum of **50 µL** of each patient sample into an empty sterile tube. Mix by gently pipetting up and down.
4. To each well containing 30 µL of rehydrated master mix, add **10 µL of pooled direct sample**.
5. Affix the appropriate optical caps or optical plate seals.
6. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.

SARS-CoV-2 (N1, RdRp), RNase P, IAC Extraction-Free PCR - Multi-Platform Open System PCR Reagents

RT-PCR Set-Up Extracted Sample

BioGX Product 450-085-XMP is recommended for use with swab samples collected in 1 or 3 mL collection media (i.e. Copan ESwab™, Copan UTM®, UVT, VTM, or saline). For validated magnetic bead or silica column nucleic acid extraction method, pipette appropriate volume of sample into the extraction tube/plate and proceed with protocol as per manufacturer's instructions for use.

1. Transfer **400 µL** of **molecular grade water** to one vial of lyophilized BioGX Xfree SARS-CoV-2 reagents. Mix by gently pipetting up and down with 1000 µL pipet tip. (IMPORTANT: Keep rehydrated master mix in a cold block or on ice if set-up 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).
2. Transfer **10 µL** of **rehydrated master mix** to the bottom of **40 empty wells** (8-tube PCR strips or 96-well PCR plate).
3. To each well containing 10 µL of rehydrated master mix, add **5 µL** of **extracted sample**.
4. Affix the appropriate optical caps or optical plate seals.
5. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.

Table 2. Interpretation of sample results.

N1 gene	RdRp gene	RNase P	IAC	Result Interpretation
+	+	+	+/-	SARS-CoV-2 POSITIVE
+	-	+	+/-	SARS-CoV-2 POSITIVE
-	+	+	+/-	SARS-CoV-2 POSITIVE
-	-	+	+	SARS-CoV-2 NEGATIVE
-	-	-	+	Indeterminant
-	-	+	-	Indeterminant
-	-	-	-	Indeterminant

SARS-CoV-2 (N1, RdRp), RNase P, IAC Extraction-Free PCR - Multi-Platform Open System PCR Reagents

Preparation of BioGX External Control for Direct or Extracted Sample Testing

Applied Biosystems or Bio-Rad platforms

Positive Control [BioGX SARS-CoV-2 (N1 and RdRp)]

Preparation of BioGX SARS-CoV-2 Synthetic Nucleocapsid Phosphoprotein Gene Region (N1) (part number: 720-0206), SARS-CoV-2 RNA-Dependent RNA Polymerase (RdRp) (Part number: 720-0209) and external control.

A single BioGX lyophilized SARS-CoV-2 (N1) and SARS-CoV-2 (RdRp) control bead containing 100,000 copies/bead should be serially diluted as described in the procedure below to achieve a final concentration of 10,000 copies/mL (=10 copies/ μ L).

1. Rehydrate one BioGX lyophilized SARS-CoV-2 (N1) control bead and one BioGX lyophilized SARS-CoV-2 (RdRp) control bead with 100 μ L of molecular grade water each. Slowly pipette up and down 5 times to homogenize and discard pipette tip. This will be the SARS-CoV-2 (N1) and SARS-CoV-2 (RdRp) control bead stock solutions.
2. With a fresh pipette tip, prepare two microcentrifuge tubes for serial dilution by adding 90 μ L of molecular grade water to each. Label the tube caps with **"N1-10" or "RdRp-10"** and **"N1-100" or "RdRp-100"** to represent the 1/10 dilution and 1/100 dilutions of SARS-CoV-2 (N1) and SARS-CoV-2 (RdRp) stock solutions, respectively.
3. With a fresh pipette tip, transfer 10 μ L of the SARS-CoV-2 (N1) and SARS-CoV-2 (RdRp) stock solutions to the first microcentrifuge tube labeled **"N1-10" or "RdRp-10"**. Slowly pipette up and down 5 times to homogenize then discard the pipette tip.
4. With a fresh pipette tip, transfer 10 μ L of the **"N1-10" or "RdRp-10"** dilution to the second microcentrifuge tube labeled **"N1-100" or "RdRp-100"**. Slowly pipette up and down 5 times to homogenize then discard the pipette tip. The microcentrifuge tube labeled **"N1-100" or "RdRp-100"** now contains SARS-CoV-2 (N1) or SARS-CoV-2 (RdRp) control at 10,000 copies/mL (=10 copies/ μ L).

Add **5 μ L** of SARS-CoV-2 (N1) control (50 copies/PCR reaction) from the microcentrifuge tube labeled **"N1-100"** to each positive control well position(s) containing dispensed master mix.

Add **5 μ L** of SARS-CoV-2 (RdRp) control (50 copies/PCR reaction) from the microcentrifuge tube labeled **"RdRp-100"** to each positive control well position(s) containing dispensed master mix.

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Negative Control (No Template) - Add 5 µL of molecular grade water to (1) well position containing dispensed master mix.

Table 3. Interpretation of BioGX external controls when run on Applied Biosystems or Bio-Rad platforms.

Control Type	Applicability for Monitoring	N1 Gene	N1 Expected Ct	RdRp Gene	RdRp Expected Ct	RNase P	RNase P Expected Ct	IAC	IAC Expected Ct
Negative Control - Addition of 5 µL Water	Reagent and/or environmental contamination	-	Not Detected	-	Not Detected	-	Not Detected	+	Detected
N1 Positive Control - BioGX Synthetic Template Control	Substantial reagent failure including primer and probe integrity	+	Detected	-	Not Detected	-	Not Detected	+	Detected
RdRp Positive Control - BioGX Synthetic Template Control	Substantial reagent failure including primer and probe integrity	-	Not Detected	+	Detected	-	Not Detected	+	Detected

Preparation of BioGX External Controls

BioGX pixl.16

Positive Control [BioGX SARS-CoV-2 (N1) and SARS-CoV-2 (RdRp)] - Each lyophilized template bead should be rehydrated with **500 µL** of molecular grade water and mixed by pipetting up and down 5 times. Add **5 µL** of the positive control BioGX SARS-CoV-2 (N1) or SARS-CoV-2 (RdRp) to (1) well position containing dispensed master mix.

Negative Control (No Template) - Addition of **5 µL** of molecular grade water to (1) well position containing dispensed master mix.

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Table 4. Interpretation of BioGX external controls when run on BioGX pixl.16 platform

Control Type	Applicability for Monitoring	N1 Gene	N1 Expected Ct	RdRp Gene	RdRp Expected Ct	RNase P	RNase P Expected Ct	IAC	IAC Expected Ct
Negative Control - Addition of 5 µL Water	Reagent and/or environmental contamination	-	Not Detected	-	Not Detected	-	Not Detected	+	Detected
N1 Positive Control - BioGX Synthetic Template Control	Substantial reagent failure including primer and probe integrity	+	Detected	-	Not Detected	-	Not Detected	+	Detected
RdRp Positive Control - BioGX Synthetic Template Control	Substantial reagent failure including primer and probe integrity	-	Not Detected	+	Detected	-	Not Detected	+	Detected

Sample Types Collections without Human Nucleic Acid Content

Please note that sample types devoid of human cells and/or human nucleic acids will not generate an RNase P amplification curve. For such samples being analyzed with direct sample addition, it is recommended to spike 5,000 copies of BioGX RNase P template control. For samples that will be extracted with validated silica column or magnetic bead extraction systems, spiking template control at a copy number to yield 5,000-10,000 copies of RNase P per 5 µL of purified sample (e.g., spike 1×10^5 copies per sample extracted and eluted in 50 µL to support sample template addition that will contain 10,000 copies of BioGX synthetic RNase P RNA).

ASSAY PERFORMANCE

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

SARS-CoV-2 (N1, RdRp), RNase P, IAC Extraction-Free PCR - Multi-Platform Open System PCR Reagents

REFERENCES









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13. 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).
14. BioGX pixl.16 Real-Time PCR Platform Instructions for Use (refer to the latest version), BioGX, Inc., Birmingham, Alabama, USA.

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

**SARS-CoV-2 (N1, RdRp), RNase P, IAC
Extraction-Free PCR - Multi-Platform
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Rev. #	Effective Date	Summary of Changes
01	12 APR 2023	Initial Release.

SYMBOLS

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



BioGX

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