BioGX
Molecular Made Easy

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

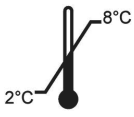
REF **450-065-XMP**

Σ 104 Reactions

Product Insert

For Research Use Only: Not for use in diagnostic procedures

For use with ABI QuantStudio™ 5, Bio-Rad CFX96 Touch™

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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.

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) and Bio-Rad CFX96 Touch™ (Maestro software version 1.1 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]), 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.

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

1. ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™
   REF 450-065-XMP

   OSR for ABI and Bio-Rad Platforms (450-065-XMP) contain all PCR primers, probes, enzymes, dNTPs, $MgCl_2$, 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.

BioGX REF: 450-065-XMP
Platform(s): ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™

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.

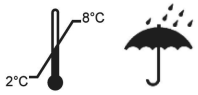
EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BioGX Lyophilized Positive Control Template DNA Beads (10^5 copies/bead)
  - SARS-CoV-2 Nucleocapsid phosphoprotein gene (N1) (Part number 720-0206)
  - RNase P (Part number 720-0208)
- BioGX Molecular Grade Water or equivalent
  - BioGX Rehydration Water (Part number: 800-0035-12)
- BioGX BioGX Lyophilized Far Red Dye Calibration Kit for Applied Biosystems QuantStudio 5 (Part number 720-0219)
- 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)
- 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)

WARNINGS AND PRECAUTIONS

- For research use only. Not intended for use in diagnostic procedures.
- 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 M29[4] and in Biosafety in Microbiological and Biomedical Laboratories[5].
- This test has been optimized only with the Applied Biosystems QuantStudio™ 5 and Bio-Rad CFX96 Touch™ 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.

STORAGE REQUIREMENTS AND RECOMMENDATIONS



Reagents are stable at ambient temperature (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 450-065-XMP[a].

Optical Channel (Fluorophore Equivalent)	**Target**
FAM	RNase P
HEX	Unused
Texas Red	SARS-CoV-2 (N1)
Cy5	Unused
Cy5.5 [a]	IAC

[a]***When using the Applied Biosystems Quantstudio 5 instrument, the BioGX Lyophilized Far Red Dye Calibration Kit for Applied Biosystems QuantStudio 5 (Part number 720-0219) will be needed to add this dye to the instrument dye library. Please refer to the Product Insert of 720-0219 available on www.biogx.com for instructions and further details[8].***

QUALITY CONTROL, REAGENT PREPARATION AND EXTRACTION TESTING PARAMETERS

ABI and Bio-Rad Platforms (BioGX REF: 450-065-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. Bio-Rad CFX96 Touch™ (Maestro software version 1.1 or later)

BioGX's most current ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™ 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 manual[6] for uploading instructions. Please refer to the Bio-Rad CFX96 Touch™ user manual[7] for uploading instructions.

RT-PCR Set-Up Direct Individual Sample

BioGX Product 450-065-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.

RT-PCR Set-Up Direct Pooled Sample

BioGX Product 450-065-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.

RT-PCR Set-Up Extracted Sample

BioGX Product 450-065-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.

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 x 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.

Table 2. Interpretation of sample results.

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

REFERENCES

1. US Centers for Disease Control and Prevention. 2020. 2019-Novel coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes. https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf
2. US Centers for Disease Control and Prevention. 2020. Revision to Test Instructions CDC 2019 Novel Coronavirus (nCoV) Real-Time RT-PCR Diagnostic Panel (EUA200001). https://www.aphl.org/Materials/Signed_CDC_Letter_to_PHLs-N3_Removal_Instructions_26eb2020.pdf
3. US Centers for Disease Control and Prevention. 2020. 2019-Novel coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes. CDC-006-00019, Revision: 02. https://www.fda.gov/media/134922/download
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. QuantStudio™ Design and Analysis software User Guide, ThermoFisher Scientific, Waltham, Massachusetts, USA (Refer to the latest version).
7. CFX96 Touch, CFX96 Touch Deep Well, CFX Connect, and CFX384 Touch Systems Instruction Manual (refer to the latest version), Bio-Rad Laboratories, Inc., Hercules,mCalifornia, USA (Refer to the latest version).
8. BioGX Lyophilized Far Red Dye Calibration Kit for Applied Biosystems QuantStudio™ 5 Real-Time PCR system Product number series 720-0219 (www.biogx.com)

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

Rev. #	Effective Date	Summary of Changes
04	18 NOV 2025	Updated research use only text.
03	21 JAN 2022	Update of result interpretation table 2, Update to include reference to BioGX Far Red Dye Calibration Kit for use with Applied Biosystems Quantstudio 5
02	11 AUG 2021	Update branding and storage conditions.
01	02 JUL 2021	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

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