



BioGX

Molecular Made Easy

Xfree COVID-19 Direct RT-PCR

REF 500-003-XMP



104 Extraction-Free Sample-Direct Tests

Instructions For Use

For *In Vitro* Diagnostic Use

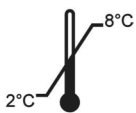
For Emergency Use Authorization (EUA) Only

For use with:

Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well and 384-well) and 7500 Fast Dx
Bio-Rad CFX96 Touch™ and CFX384 Touch™

Bio Molecular Systems Mic Instrument

BioGX pixl.16



IVD

R_x Only

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INTENDED USE

The BioGX Xfree™ COVID-19 Direct RT-PCR (Xfree™ COVID-19) assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 virus in nasopharyngeal, anterior nasal, mid-turbinate, and oropharyngeal swab specimens, nasopharyngeal wash/aspirate or nasal aspirates obtained from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of the disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Xfree™ COVID-19 assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR *in vitro* diagnostic procedures. The Xfree™ COVID-19 assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF THE TEST

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the novel betacoronavirus that causes the COVID-19 respiratory disease. The COVID-19 respiratory disease is transmitted amongst infected humans within populations via respiratory droplets from talking, coughing, and sneezing^{1,2}. Symptoms of COVID-19 range from mild illness (dry cough, fatigue, fever, and shortness of breath) to severe illness and death¹.

COVID-19 was first identified on December 31, 2019, amongst patients suffering from pneumonia in Wuhan, China^{1,2}. SARS-CoV-2 is the seventh coronavirus identified as a human pathogen that likely evolved within an animal host or within humans following zoonotic transfer³.

As of September 29, 2020, the WHO has confirmed 33,034,598 infections and 996,342 COVID-19 related deaths globally². The elderly, immunocompromised, and those with cardiovascular disease, diabetes, and chronic respiratory disease have been shown to be at higher risk for severe illness^{1,2}. Ahead of global vaccination efforts, patient testing and social distancing measures mandated by world governments have proven to be the only way to stem transmission rates.

BioGX Xfree™ COVID-19 assay is a real-time RT-PCR test that utilizes primer and probe sequences based on the United States Centers for Disease Control and Prevention (US CDC) assay for detection of SARS-CoV-2 (N1 gene region) and human RNase P^{4,5,6}. Additionally, a non-naturally occurring single-stranded RNA has been integrated into the lyophilized format to serve as an internal amplification control.

The PCR assay is a multiplex test that utilizes a BioGX Sample-Ready™ lyophilized reagent provided in 2 mL vials. Each vial contains all PCR components such as primers, probes, enzymes, dNTPs, MgCl₂, Internal Amplification Control RNA (IAC), and buffers required for real-time RT-PCR-based testing. The assay has been validated utilizing two different workflows, the direct workflow in which patient sample is added directly to the reconstituted BioGX RT-PCR mix without any pre-processing or extraction steps, and the non-direct workflow that requires nucleic acid extraction using the QIAGEN QIAamp Viral RNA Mini Kit. Each lyophilized vial contains enough material to test 104 direct patient samples or 160 extracted/purified nucleic acid samples with one the following platforms:

- **Applied Biosystems QuantStudio™ 5** (0.2 mL 96-well) utilizing Design & Analysis software version 1.5.1
- **Applied Biosystems QuantStudio™ 5 (384-well)** utilizing Design & Analysis software version 1.5.1
- **Applied Biosystems 7500 Fast Dx** utilizing SDS software version 1.4.1.
- **Bio-Rad CFX96 Touch™** utilizing Maestro software version 1.1
- **Bio-Rad CFX384 Touch™** utilizing Maestro software version 1.1
- **Bio Molecular Systems Mic Instrument** utilizing Mic PCR software version 2.8
- **BioGX pixl.16** utilizing software version 1.6.9

Applied Biosystems QuantStudio 5 trademark is the property of ThermoFisher Scientific.
Bio-Rad CFX96 Touch and Bio-Rad CFX384 Touch trademarks are the property of Bio-Rad Laboratories.

PRINCIPLES OF THE PROCEDURE

The BioGX Xfree™ COVID-19 assay is to be used with the Applied Biosystems QuantStudio™ 5 (96-well and 384-well), Applied Biosystems 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™, Bio Molecular Systems Mic or BioGX pixl.16 platforms with direct patient sample or extracted patient sample. The template RNA is reverse

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- RNA Control (External Control):
 - ZeptoMetrix (catalog number: NATSARS(CoV2)-ST) or
 - BioGX Synthetic Nucleocapsid Phosphoprotein Gene Region (N1) (catalog no. 720-0206)
- Sterile DNase/RNase free 1.5 mL snap cap tubes
- BioGX Molecular Grade Water or equivalent
 - BioGX Rehydration Water Part number: 800-0035-12
- Sterile swab collection device appropriate for nasopharyngeal, anterior nasal, mid-turbinate or oropharyngeal swab collections
- Copan universal transport media (UTM®) collection kit
- Copan ESwab™ collection kit
- BD™ universal viral transport (UVT) collection kit
- Sterile viral transport media (VTM)
- Sterile saline (0.85% NaCl)
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent
- Disposable nitrile gloves
- Calibrated Manual or Repeater Pipettes capable of accurately dispensing: 2 µL, 10 µL, 100 µL, 1000 µL
- Sterile DNase/RNase free filter tip pipette tips
- Optional if using extraction: QIAGEN QIAamp Viral RNA Mini Kit (REF 52906)
 - Centrifuge compatible with QIAGEN extraction kit tubes
- Applied Biosystems QuantStudio 5 (0.2 mL 96-well) with Design & Analysis software v1.5.1 and 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 QuantStudio 5 (384-well) with Design & Analysis software v1.5.1 and consumables.
 - Thermo Fisher MicroAmp® Endura Plate Optical 384-well Clear Reaction Plate with Barcode (Product number: 4483258 or equivalent)
 - Thermo Fisher Optical plate seal (Product number: AB0558 or equivalent)
- Applied Biosystems 7500 Fast Dx (0.1 mL) with SDS software v1.4.1 and 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 with Maestro software v1.1 and 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 with software v1.1 and consumables
 - Bio-Rad 384-well plates (catalog no. HSP3905)
 - Bio-Rad 384-well plate sealing film, optical (catalog no. MSB1001)
- Bio Molecular Systems Mic PCR Instrument with Mic PCR software version 2.8 and consumables
 - Mic PCR Instrument clear reaction tubes and caps (catalog no. 60653)
 - Mic PCR Instrument reaction tubes loading block (catalog no. 80418)
 - Mic PCR Instrument reaction tubes capping tool (catalog no. 90690)
- BioGX pixl.16 instrument with software version 1.6.9 and consumables
 - BioGX pixl.16 (catalog no. 650-016-PXL)
 - TempAssure® PCR 8-Tube clear strips, Att. Optical Caps (catalog no. 010-280-ETS)

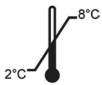
WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- For prescription use only.
- For use under an Emergency Use Authorization (EUA) only.
- The Xfree™ COVID-19 assay has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by authorized laboratories; laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.
- The Xfree™ COVID-19 assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- If infection with SARS-CoV-2 is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Specimen processing should be performed in accordance with national biological safety regulations.
- Treat all biological specimens as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29⁷ and in Biosafety in Microbiological and Biomedical Laboratories⁸.



- Performance characteristics of this test have been established only with the specimen types listed in “Intended Use” section. The performance of this assay with other specimen types or samples has not been evaluated.
- 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 specimens 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.

REAGENT STORAGE AND STABILITY



- Reagents have been tested to demonstrate optimal performance when stored properly at 2-8°C and consumed by the expiration date on the kit label. Expiration dating of reagent pouch is 15 months from date of manufacture.



- Avoid exposing the reagents (lyophilized or rehydrated) to direct sunlight or long-term ambient lighting.

- **Discard unused rehydrated master mix if not used immediately. Re-use may result in contamination and false results.**



- If pouch is opened by the user but lyophilized reagent is not rehydrated, avoid exposure of lyophilized reagent to moisture.

SPECIMEN COLLECTION, STORAGE, AND HANDLING

Wear gloves when handling Copan Universal Transport Media (UTM), BD Universal Viral Transport (UVT), Copan ESwab™, or Saline collected specimens. If gloves come in contact with the specimen, immediately change gloves to prevent contamination of other specimens.

Collection: Nasopharyngeal/Oropharyngeal swab specimens should be collected according to the manufacturer's instructions for use. Swab specimens should be collected using only swabs with a synthetic tip, and an aluminum or plastic shaft. Calcium alginate swabs are not acceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing either 3 mL of viral transport media (UTM/VTM) or 1 mL of modified liquid Amies media (ESwab™).

Nasopharyngeal swab (NP): Insert a swab into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.

Oropharyngeal swab (e.g., throat swab, OP): Swab the posterior pharynx, avoiding the tongue.

Anterior Nasal Swab (NS): Using a flocced or spun polyester swab, insert the swab at least 1 cm (0.5 inch) inside the nostril (naris) and firmly sample the nasal membrane by rotating the swab and leaving in place for 10 to 15 seconds.

Sample both nostrils with same swab.

Storage: Specimens may be stored at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.

Shipping: Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Store specimens at 2-8°C and ship overnight to the lab on ice pack. Specimens frozen at -70°C are shipped overnight to the lab on dry ice.

Additional information on packing, shipping, and transporting of specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

For further guidance on specimen collection, storage and handling from patients suspected to be infected with SARS-CoV-2, reference the following CDC Interim Guidelines: <https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html>.

INSTRUCTIONS FOR USE

Instrument Templates/Protocols and Programming

Please refer to Appendix H for appropriate EUO labeling of instrument platforms.

Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well)

(Operating software: Design & Analysis Software v1.5.1)

Install the BioGX Protocol on the Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well)

It will be necessary to import the Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well) template. The most current Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well) template is available for download at www.biogx.com. Follow the 'Education Center' tab in the navigation menu at the top of the home page. Select "US Product Documents". Follow the "Xfree™ Direct RT-PCR Documents" tab. Choose the appropriate product number and follow the "Run Files" link to the platform of choice. Download the Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well) template. Alternatively, the Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well) template can also be obtained by emailing BioGX at covid19@biogx.com. To manually program the thermocycler, please refer to **Appendix A**. For further instrument information, please refer to the Applied Biosystems QuantStudio™ 5 user manual⁹.

Applied Biosystems QuantStudio™ 5 (384-well)

(Operating software: Design & Analysis Software v1.5.1 or later)

Install the BioGX Protocol on the Applied Biosystems QuantStudio™ 5 (384-well)

It will be necessary to import the Applied Biosystems QuantStudio™ 5 (384-well) template. The most current Applied Biosystems QuantStudio™ 5 (384-well) template is available for download at www.biogx.com. Follow the "Education Center" tab in the navigation menu at the top of the home page. Depending on your geographical location, select "U.S. Product Documents" or "Int. Product Documents". Follow the "Xfree™ Direct RT-PCR Documents" tab. Choose the appropriate product number and follow the "Run

Files" link to the platform of choice. Download the Applied Biosystems QuantStudio™ 5 (384-well) template. Alternatively, the Applied Biosystems QuantStudio™ 5 (384-well) template can also be obtained by emailing BioGX at covid19@biogx.com. To manually program the thermocycler, please refer to **Appendix B**. For further instrument information, please refer to the Applied Biosystems QuantStudio™ 5 (384-well) user manual⁹.

Applied Biosystems 7500 Fast Dx

(Operating software: SDS Software v1.4.1)

Install the BioGX Protocol on the Applied Biosystems 7500 Fast Dx

It will be necessary to import the Applied Biosystems 7500 Fast Dx template. The most current Applied Biosystems 7500 Fast Dx template is available for download at www.biogx.com. Follow the "Education Center" tab in the navigation menu at the top of the home page. Select "US Product Documents". Follow the "Xfree™ Direct RT-PCR Documents" tab. Choose the appropriate product number and follow the "Run Files" link to the platform of choice. Download the Applied Biosystems 7500 Fast Dx template. Alternatively, the Applied Biosystems 7500 Fast Dx template can also be obtained by emailing BioGX at covid19@biogx.com. To manually program the thermocycler, please refer to **Appendix C**. For further instrument information, please refer to the Applied Biosystems 7500 Fast Dx user manual¹⁰.

Bio-Rad CFX96 Touch™

(Operating software: Maestro™ 1.1)

Install the BioGX Protocol on the Bio-Rad CFX96 Touch™

It will be necessary to import the Bio-Rad CFX96 Touch™ Protocol and Plate files. The most current versions of the Bio-Rad CFX96 Touch™ Protocol and Plate files are available for download on www.biogx.com. Follow the "Education Center" tab in the navigation menu at the top of the home page. Select "US Product Documents". Follow the "Xfree™ Direct RT-PCR Documents" tab. Choose the appropriate product number and follow the "Run Files" link to the platform of choice. Download the Bio-Rad CFX96 Touch™ Protocol and Plate files. Alternatively, the Bio-Rad CFX96 Touch™ Protocol and Plate files can also be obtained by emailing BioGX at covid19@biogx.com. To manually program the thermocycler, please refer to **Appendix D**. For further instrument information, please refer to the Bio-Rad CFX96 Touch™ user manual¹¹.

Bio-Rad CFX384 Touch™

(Operating software: Maestro™ 1.1)

Install the BioGX Protocol on the Bio-Rad CFX384 Touch™

It will be necessary to import the Bio-Rad CFX384 Touch™ Protocol and Plate files. The most current versions of the Bio-Rad CFX384 Touch™ Protocol and Plate files are available for download on www.biogx.com. Follow the "Education Center" tab in the navigation menu at the top of the home page. Select "US Product Documents". Follow the "Xfree™ Direct RT-PCR Documents" tab. Choose the appropriate product number and follow the "Run Files" link to the platform of choice. Download the Bio-Rad CFX384 Touch™ Protocol and Plate files. Alternatively, the Bio-Rad CFX384 Touch™ Protocol and Plate files can also be obtained by emailing BioGX at covid19@biogx.com. To manually program the thermocycler, please refer to **Appendix E**. For further instrument information, please refer to the Bio-Rad CFX384 Touch™ user manual¹¹.

BioGX pixl.16

(Operating software: version 1.6.9)

Install the BioGX Protocol on the BioGX pixl.16

It will be necessary to import the BioGX pixl.16 template. The most current versions of the BioGX pixl.16 template are available for download on www.biogx.com. Follow the "Education Center" tab in the navigation menu at the top of the home page. Depending on your location, select "U.S. Product Documents" or "Int. Product Documents". Follow the "Xfree™ Direct RT-PCR Documents" tab. Choose the appropriate product number and follow the "Run Files" link to the platform of choice. Download the BioGX pixl.16 template. Alternatively, the BioGX pixl.16 template can also be obtained by emailing BioGX at covid19@biogx.com. To manually program the thermocycler, please refer to **Appendix F**. For further instrument information, please refer to the BioGX pixl.16 user manual¹².

Bio Molecular Systems Mic Instrument

(Operating software: version 2.8)

Install the BioGX Protocol on the Mic Instrument

It will be necessary to import the Mic Instrument template. The most current versions of the Mic Instrument template are available for download on www.biogx.com. Follow the "Education Center" tab in the navigation menu at the top of the home page. Depending on your location, select "U.S. Product Documents" or "Int. Product Documents". Follow the "Xfree™ Direct RT-PCR Documents" tab. Choose the appropriate product number and follow the "Run Files" link to the platform of choice. Download the Mic Instrument. Alternatively, the Mic Instrument template can also be obtained by emailing BioGX at info@biogx.com or eu@biogx.com. To manually program the thermocycler, please refer to **Appendix G**. For further instrument information, please refer to the Bio Molecular Systems Mic Instrument user manual¹³.

RT-PCR SET-UP - DIRECT PATIENT SAMPLE

Follow the “RT-PCR Set-up - Direct Patient Sample” section below for the appropriate real-time PCR platform (i.e. QuantStudio™ 5 (96-well and 384-well), 7500 Fast Dx, CFX96 Touch™, CFX384 Touch™, BioGX pixl or Bio Molecular Systems Mic).

Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well)



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 26 samples to be tested by direct sample addition.
4. 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 should be discarded.**)
5. Transfer **15 µL of rehydrated master mix** to the bottom of **26 empty wells** (8-tube PCR strips or 96-well PCR plate).
6. To each well containing 15 µL of rehydrated master mix, add **5 µL of direct patient sample**.
7. Affix the appropriate optical caps or optical plate seals.
8. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.
9. Load 8-tube PCR strips and/or 96-well PCR plates into the real-time PCR platform and start run. Avoid unnecessary delay once tubes/plates are loaded.
10. Refer to **Table 2** for result interpretation.

Note: For Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well) template file for Direct Patient Sample addition (500003XMPDirectQS5v2.edt), please refer to the section: **“Instrument Templates and Programming”**: **Install the BioGX Protocol on the Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well)** for download instructions. To manually program the thermocycler, please refer to **Appendix A**.

Applied Biosystems QuantStudio™ 5 (384-well)



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 384-well PCR plates.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 26 samples to be tested by direct sample addition.
4. 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 is not fully dispensed, discard unused volume).
5. Transfer **15 µL of rehydrated master mix** to the bottom of **26 empty wells** (384-well PCR plate).
6. To each well containing 15 µL of rehydrated master mix, add **5 µL of direct patient sample**.
7. Affix the instrument platform appropriate optical caps or optical plate seals.
8. Pulse spin the sealed PCR plate to mix and bring liquid to the bottom.
9. Load 384-well PCR plates into the real-time PCR platform and start run. Avoid unnecessary delay once tubes/plates are loaded.
10. Refer to **Table 2** for result interpretation.

Note: For Applied Biosystems QuantStudio™ 5 (384-well) template file for Direct Patient Sample addition (500003XMPDirectQS5_384Rev01.edt), please refer to the section: **“Instrument Templates and Programming”**: **Install the BioGX Protocol on the Applied Biosystems QuantStudio™ 5 (384-well)** for download instructions. To manually program the thermocycler, please refer to **Appendix B**.

RT-PCR SET-UP - DIRECT PATIENT SAMPLE

Applied Biosystems 7500 Fast Dx



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 26 samples to be tested by direct sample addition.
4. 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 should be discarded.**
5. Transfer **15 µL** of **rehydrated master mix** to the bottom of **26 empty wells** (8-tube PCR strips or 96-well PCR plate).
 6. To each well containing 15 µL of rehydrated master mix, add **5 µL of direct patient sample**.
 7. Affix the appropriate optical caps or optical plate seals.
 8. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.
 9. Load 8-tube PCR strips and/or 96-well PCR plates into the real-time PCR platform and start run. Avoid unnecessary delay once tubes/plates are loaded.
 10. Refer to **Table 2** for result interpretation.

Note: For Applied Biosystems 7500 Fast Dx template file for Direct Patient Sample addition (500003XMPDirect7500Dx.sdt), please refer to the section: **“Instrument Templates and Programming”**: **Install the BioGX Protocol on the Applied Biosystems 7500 Fast Dx** for download instructions. To manually program the thermocycler, please refer to **Appendix C**.

RT-PCR SET-UP - DIRECT PATIENT SAMPLE

Bio-Rad CFX96 Touch™

1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 26 samples to be tested by direct sample addition.
4. 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 should be discarded.**)
5. Transfer **15 µL** of **rehydrated master mix** to the bottom of **26 empty wells** (8-tube PCR strips or 96-well PCR plate).
6. To each well containing 15 µL of rehydrated master mix, add **5 µL of direct patient sample**.
7. Affix the appropriate optical caps or optical plate seals.
8. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.

9. When CFX96 Touch™ run is completed, threshold setting update will need to be completed as follows:
 - a. Open the “Data Analysis” window for your specific run
 - b. To apply threshold settings for FAM-channel, uncheck CalRed 610-channel and Quasar 670-channel and be sure “**FAM**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for FAM will apply to the experiment.
 - c. To apply threshold settings for CalRed 610-channel, uncheck FAM-channel and Quasar 670-channel and be sure “**CalRed 610**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for CalRed 610 will apply to the experiment
 - d. To apply threshold settings for Quasar 670-channel, uncheck FAM-channel and CalRed 610-channel and be sure “**Quasar 670**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “**Baseline Threshold**”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for Quasar 670 will apply to the experiment
10. Refer to **Table 2** for result interpretation.

Note: For Bio-Rad CFX96 Touch™ protocol and plate files for Direct Patient Sample addition (500003XMPDirectCFX96v2.prcl and 500003XMPDirect_plateCFX96v2.pltd), please refer to the section: “**Instrument Templates and Programming**”: **Install the BioGX Protocol on the Bio-Rad CFX96 Touch™** for download instructions. To manually program the thermocycler, please refer to **Appendix D**.

RT-PCR SET-UP - DIRECT PATIENT SAMPLE

Bio-Rad CFX384 Touch™

1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 384-well PCR plates.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 26 samples to be tested by direct sample addition.
4. 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 should be discarded.**)
5. Transfer **15 µL of rehydrated master mix** to the bottom of **26 empty wells** (384-well PCR plate).
6. To each well containing 15 µL of rehydrated master mix, add **5 µL of direct patient sample**.
7. Affix the appropriate optical caps or optical plate seals.
8. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.
9. When CFX384 Touch™ run is completed, threshold setting update will need to be completed as follows:
 - a. Open the “Data Analysis” window for your specific run
 - b. To apply threshold settings for FAM-channel, uncheck CalRed 610-channel and Quasar 670-channel and be sure “**FAM**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for FAM will apply to the experiment.
 - d. To apply threshold settings for CalRed 610-channel, uncheck FAM-channel and Quasar 670-channel and be sure “**CalRed 610**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**250**”
 - iii. Click “OK” and the threshold for CalRed 610 will apply to the experiment
 - e. To apply threshold settings for Quasar 670-channel, uncheck FAM-channel and CalRed 610-channel and be sure “**Quasar 670**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “**Baseline Threshold**”

- ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “500”
 - iii. Click “OK” and the threshold for Quasar 670 will apply to the experiment
10. Refer to **Table 2** for result interpretation.

Note: For Bio-Rad CFX384 Touch™ protocol and plate files for Direct Patient Sample addition (500003XMPDirectCFX384.prcl and 500003XMPDirect_plateCFX384.pltd), please refer to the section: “**Instrument Templates and Programming**”: **Install the BioGX Protocol on the Bio-Rad CFX384 Touch™** for download instructions. To manually program the thermocycler, please refer to **Appendix E**.

RT-PCR SET-UP - DIRECT PATIENT SAMPLE

BioGX pixl.16



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 8-tube PCR strips.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 26 samples to be tested by direct sample addition.
4. 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 is not fully dispensed, discard unused volume).
5. Transfer **15 µL of rehydrated master mix** to the bottom of **1 to 16 empty wells** (8-tube PCR strips).
6. To each PCR tube containing 15 µL of rehydrated master mix, add **5 µL of direct patient sample**.
7. Affix the optical caps.
8. Pulse spin the sealed 8-tube PCR strips to mix and bring liquid to the bottom.
9. Load 8-tube PCR strips into the real-time PCR platform and start run. Avoid unnecessary delay once tubes are loaded.
10. Refer to **Table 2** for result interpretation.

Note: For BioGX pixl.16 template file for Direct Patient Sample addition (500003XMPDirectpixlRev01.json), please refer to the section: “**Instrument Templates and Programming**”: **Install the BioGX Protocol on the BioGX pixl.16** for download instructions. To manually program the thermocycler, please refer to **Appendix F**.

RT-PCR SET-UP - DIRECT PATIENT SAMPLE

Bio Molecular Systems Mic Instrument



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of PCR tubes.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 26 samples to be tested by direct sample addition.
4. 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 is not fully dispensed, discard unused volume).
5. Transfer **15 µL of rehydrated master mix** to the bottom of **26 empty PCR tubes**. To each PCR tube containing 15 µL of rehydrated master mix, add **5 µL of direct patient sample**.
6. Affix the reaction tube caps.
7. Pulse spin the sealed PCR tubes to mix and bring liquid to the bottom.
8. Load the reaction tubes into the real-time PCR platform and start run. Avoid unnecessary delay once tubes are loaded.
9. Refer to **Table 2** for result interpretation.

Note: For Bio Molecular Systems Mic Instrument template file for Direct Patient Sample addition (500003XMPDirectMICRev01.mictemplate), please refer to the section: **“Instrument Templates and Programming”**: **Install the BioGX Protocol on the Bio Molecular Systems Mic Instrument** for download instructions. To manually program the thermocycler, please refer to **Appendix G**.

RT-PCR SET-UP - EXTRACTED PATIENT SAMPLE

Follow the “RT-PCR Set-up - Extracted Patient Sample” section below for the appropriate real-time PCR platform (i.e. QuantStudio™ 5 (96-well and 384-well), 7500

Fast Dx, CFX96 Touch™, CFX384 Touch™, BioGX pixl or Bio Molecular Systems Mic). For the validated QIAGEN QIAamp Viral RNA Mini Kit extraction method (manual), pipette 140µL of specimen into the extraction tube/plate and proceed with the protocol as per the manufacturer's instructions for use (Purification of Viral RNA (Spin Protocol)). Elute each sample in 50µL RNase-free water. After extraction and elution, add **5 µL** of the eluate to the RT-PCR master mix.

Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well)

1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 40 samples to be tested using purified nucleic acid samples.
4. 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 should be discarded.**)
5. Transfer **10 µL of rehydrated master mix** to the bottom of **40 empty wells** (8-tube PCR strips or 96-well PCR plate).
6. To each well containing 10 µL of rehydrated master mix, add **5 µL of extracted patient sample**.
7. Affix the appropriate optical caps or optical plate seals.
8. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.
9. Load 8-tube PCR strips and/or 96-well PCR plates into the real-time PCR platform and start run. Avoid unnecessary delay once tubes/plates are loaded.
10. Refer to **Table 2** for result interpretation.

Note: For Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well) template file for Extracted Patient Sample addition (500003XMPExtractQS5v2.edt), please refer to the section: “**Instrument Templates and Programming**”: **Install the BioGX Protocol on the Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well)** for download instructions. To manually program the thermocycler, please refer to **Appendix A**.

RT-PCR SET-UP - EXTRACTED PATIENT SAMPLE

Applied Biosystems QuantStudio™ 5 (384-well)



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.

2. Prepare the appropriate number of 384-well PCR plates.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is sufficient for 40 samples to be tested using purified nucleic acid samples.
4. 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 is not fully dispensed, discard unused volume).
5. Transfer **10 µL of rehydrated master mix** to the bottom of **40 empty wells** (384-well PCR plate).
6. To each well containing 10 µL of rehydrated master mix, add **5 µL of extracted patient sample**.
7. Affix the appropriate optical plate seals.
8. Pulse spin the sealed 384-well PCR plates to mix and bring liquid to the bottom.
9. Load 384-well PCR plates into the real-time PCR platform and start run. Avoid unnecessary delay once plates are loaded.
10. Refer to **Table 2** for result interpretation.

Note: For Applied Biosystems QuantStudio™ 5 (384-well) template file for Extracted Patient Sample addition (500003XMPExtractedQS5_384Rev01.edt), please refer to the section: “**Instrument Templates and Programming**”: **Install the BioGX Protocol on the Applied Biosystems QuantStudio™ 5 (384-well)** for download instructions. To manually program the thermocycler, please refer to **Appendix B**.

RT-PCR SET-UP - EXTRACTED PATIENT SAMPLE

Applied Biosystems 7500 Fast Dx

1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 40 samples to be tested using purified nucleic acid samples.
4. 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 should be discarded.**)
5. Transfer **10 µL of rehydrated master mix** to the bottom of **40 empty wells** (8-tube PCR strips or 96-well PCR plate).

6. To each well containing 10 μL of rehydrated master mix, add **5 μL of extracted patient sample**.
7. Affix the appropriate optical caps or optical plate seals.
8. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.
9. Load 8-tube PCR strips and/or 96-well PCR plates into the real-time PCR platform and start run. Avoid unnecessary delay once tubes/plates are loaded.
10. Refer to **Table 2** for result interpretation.

Note: For Applied Biosystems 7500 Fast Dx template file for Extracted Patient Sample addition (500003XMPExtract7500Dx.sdt), please refer to the section: “**Instrument Templates and Programming**”: **Install the BioGX Protocol on the Applied Biosystems 7500 Fast Dx** for download instructions. To manually program the thermocycler, please refer to **Appendix C**.

RT-PCR SET-UP - EXTRACTED PATIENT SAMPLE

Bio-Rad CFX96 Touch™



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. **DO NOT** use latex gloves.
2. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
3. Using 1000 μL pipet tip, transfer **400 μL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 40 samples to be tested using purified nucleic acid samples.
4. 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 should be discarded.**)
5. Transfer **10 μL of rehydrated master mix** to the bottom of **40 empty wells** (8-tube PCR strips or 96-well PCR plate).
6. To each well containing 10 μL of rehydrated master mix, add **5 μL of extracted patient sample**.
7. Affix the appropriate optical caps or optical plate seals.
8. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.
9. When CFX96 Touch™ run is completed, threshold setting update will need to be completed as follows:
 - a. Open the “Data Analysis” window for your specific run

- b. To apply threshold settings for FAM-channel, uncheck CalRed 610-channel and Quasar 670-channel and be sure **"FAM"** is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to "Settings" and select "Baseline Threshold"
 - ii. At the bottom of the popup window under "single threshold", select "User Defined" and enter **"500"**
 - iii. Click "OK" and the threshold for FAM will apply to the experiment.
 - c. To apply threshold settings for CalRed 610-channel, uncheck FAM-channel and Quasar 670-channel and be sure **"CalRed 610"** is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to "Settings" and select "Baseline Threshold"
 - ii. At the bottom of the popup window under "single threshold", select "User Defined" and enter **"500"**
 - iii. Click "OK" and the threshold for CalRed 610 will apply to the experiment
 - d. To apply threshold settings for Quasar 670-channel, uncheck FAM-channel and CalRed 610-channel and be sure **"Quasar 670"** is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to "Settings" and select **"Baseline Threshold"**
 - ii. At the bottom of the popup window under "single threshold", select "User Defined" and enter **"500"**
 - iii. Click "OK" and the threshold for Quasar 670 will apply to the experiment
10. Refer to **Table 2** for result interpretation.

Note: For Bio-Rad CFX96 Touch™ protocol and plate files for Extracted Patient Sample addition (500003XMPExtractCFX96v2.prcl and 500003XMPExtract_plateCFX96v2.pltd), please refer to the section: **"Instrument Templates and Programming": Install the BioGX Protocol on the Bio-Rad CFX96 Touch™** for download instructions. To manually program the thermocycler, please refer to **Appendix D**.

RT-PCR SET-UP - EXTRACTED PATIENT SAMPLE

Bio-Rad CFX384 Touch™



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 384-well PCR plates.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 40 samples to be tested using purified nucleic acid samples.
4. 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 should be discarded.**)
5. Transfer **10 µL of rehydrated master mix** to the bottom of **40 empty wells** (384-well PCR plate).
6. To each well containing 10 µL of rehydrated master mix, add **5 µL of direct patient sample**.
7. Affix the appropriate optical caps or optical plate seals.
8. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.
9. When CFX384 Touch™ run is completed, threshold setting update will need to be completed as follows:
 - a. Open the “Data Analysis” window for your specific run
 - b. To apply threshold settings for FAM-channel, uncheck CalRed 610-channel and Quasar 670-channel and be sure “**FAM**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for FAM will apply to the experiment.
 - c. To apply threshold settings for CalRed 610-channel, uncheck FAM-channel and Quasar 670-channel and be sure “**CalRed 610**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**250**”
 - iii. Click “OK” and the threshold for CalRed 610 will apply to the experiment

- d. To apply threshold settings for Quasar 670-channel, uncheck FAM-channel and CalRed 610-channel and be sure “**Quasar 670**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “**Baseline Threshold**”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for Quasar 670 will apply to the experiment
10. Refer to **Table 2** for result interpretation.

Note: For Bio-Rad CFX384 Touch™ protocol and plate files for Extracted Patient Sample addition (500003XMPExtractCFX384.prc1 and 500003XMPExtract_plateCFX384.pltd), please refer to the section: “**Instrument Templates and Programming**”: **Install the BioGX Protocol on the Bio-Rad CFX384 Touch™** for download instructions. To manually program the thermocycler, please refer to **Appendix E**.

RT-PCR SET-UP - EXTRACTED PATIENT SAMPLE

BioGX pixl.16



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of 8-tube PCR strips.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is sufficient for 40 samples to be tested using purified nucleic acid samples.
4. 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 is not fully dispensed, discard unused volume).
5. Transfer **10 µL of rehydrated master mix** to the bottom of **1 to 16 empty wells** (8-tube PCR strips).
6. To each well containing 10 µL of rehydrated master mix, add **5 µL of extracted patient sample**.
7. Affix the optical caps.
8. Pulse spin the sealed tubes to mix and bring liquid to the bottom.
9. Load 8-tube PCR strips into the real-time PCR platform and start run. Avoid unnecessary delay once tubes are loaded.
10. Refer to **Table 2** for result interpretation.

Note: For BioGX pixl.16 template file for Extracted Patient Sample addition (500003XMPExtractedpixlRev01.json), please refer to the section: “**Instrument Templates and Programming**”: **Install the BioGX Protocol on the BioGX pixl.16** for download instructions. To manually program the thermocycler, refer to **Appendix F**.

RT-PCR SET-UP - EXTRACTED PATIENT SAMPLE

Bio Molecular Systems Mic Instrument



1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Prepare the appropriate number of PCR tubes.
3. Using 1000 µL pipet tip, transfer **400 µL of molecular grade water** to one vial of lyophilized BioGX Xfree™ COVID-19 reagents. The rehydrated master mix is more than sufficient for 26 samples to be tested by direct sample addition.
4. 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 is not fully dispensed, discard unused volume).
5. Transfer **10 µL of rehydrated master mix** to the bottom of **40 empty wells** (PCR tubes).
6. To each well containing 10 µL of rehydrated master mix, add **5 µL of extracted patient sample**.
7. Affix the reaction tube caps.
8. Pulse spin the sealed PCR tubes to mix and bring liquid to the bottom.
9. Load the reaction tubes into the real-time PCR platform and start run. Avoid unnecessary delay once tubes are loaded.
10. Refer to **Table 2** for result interpretation.

Note: For Bio Molecular Systems Mic Instrument template file for extracted Patient Sample addition (500003XMPExtractedMICRev01.mictemplate), please refer to the section: “**Instrument Templates and Programming**”: **Install the BioGX Protocol on the Bio Molecular Systems Mic Instrument** for download instructions. To manually program the thermocycler, please refer to **Appendix G**.

QUALITY CONTROL

Controls to Monitor Assay Performance

CONTROL

- Each BioGX Xfree™ COVID-19 kit includes molecular primers and probes specific for the detection of the human RNase P gene, a sample processing control (SPC), in the lyophilized RT-PCR master mix. RNase P should be present and detected in each patient sample. In addition, a non-naturally occurring single-stranded RNA is integrated into the lyophilized reagent to serve as a PCR internal amplification control (IAC). The IAC should be present and detected in every control and SARS-CoV-2 negative patient sample. No external addition of the SPC or IAC is required.

External positive and negative controls are available separately for the BioGX Xfree™ COVID-19 kit. The external positive control available from BioGX (BioGX Synthetic RNA Nucleocapsid Phosphoprotein gene region (N1) (BioGX catalog no. 720-0206) is treated as if it were a patient sample and should be positive for both the COVID-19 N1 target and the IAC target. As an alternative, inactivated SARS-CoV-2 RNA available from Zeptomatrix (catalog number NATSARS(CoV2)-ST) may also be used as an external positive control and should also be positive for both the COVID-19 N1 target and the IAC target. It is recommended that one external positive control be included with each RT-PCR run of patient samples. The external negative control should consist of molecular grade water and should be positive only for the IAC target. It is recommended that one external negative control be included in each extraction batch (as applicable) and each RT-PCR 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 contamination as a result of control preparation.

Preparation of BioGX External Control for Direct or Extracted Sample Testing - Applied Biosystems, Bio-Rad, or Bio Molecular Systems platforms

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

Preparation of BioGX SARS-CoV-2 Synthetic Nucleocapsid Phosphoprotein Gene Region (N1) external control (catalog no. 720-0206):

A single BioGX lyophilized SARS-CoV-2 (N1) 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/μL).

1. Rehydrate one BioGX lyophilized SARS-CoV-2 (N1) control bead with 100 μL of molecular grade water. Slowly pipette up and down 5 times to homogenize and discard pipette tip. This will be the SARS-CoV-2 (N1) stock solution.

2. With a fresh pipette tip, prepare two microcentrifuge tubes for serial dilution by adding 90 µL of molecular grade water to each. Label the tube caps with “N1-10” and “N1-100” to represent the 1/10 dilution and 1/100 dilution of SARS-CoV-2 (N1) stock solution, respectively.
3. With a fresh pipette tip, transfer 10 µL of the SARS-CoV-2 (N1) stock solution to the first microcentrifuge tube labeled “N1-10”. Slowly pipette up and down 5 times to homogenize then discard the pipette tip.
4. With a fresh pipette tip, transfer 10 µL of the “N1-10” dilution to the second microcentrifuge tube labeled “N1-100”. Slowly pipette up and down 5 times to homogenize then discard the pipette tip. The microcentrifuge tube labeled “N1-100” now contains SARS-CoV-2 (N1) control at 10,000 copies/mL.

Add 5 µL 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.

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

Preparation of Zeptomatrix External Positive Control for Extracted or Direct Samples

Preparation of ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2 Isolate: USA-WA1/2020)
Zeptomatrix catalog number: NATSARS(COV2)-ST

ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2) stock concentration of 1,000,000 (1,000 copies/ µL) should be serially diluted to achieve a final concentration of 10,000 copies/mL (10 copies/µL) for the extracted method

1. With a fresh pipette tip, prepare **two** microcentrifuge tubes for serial dilution by adding 18 µL of molecular grade water to each tube. Label the tube caps with “N1-10” and “N1-100” to represent the 10 times dilution and 100 times dilution of stock to yield 100 copies /µL and 10 copies / µL, respectively.
2. With a fresh pipette tip, transfer 2 µL of the ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2) stock solution to the first microcentrifuge tube labeled “ZM-10”. Slowly pipette up and down 5 times to homogenize then discard the pipette tip.
3. With a fresh pipette tip, transfer 2 µL of the “N1-10” dilution to the second microcentrifuge tube labeled “N1-100”. Slowly pipette up and down 5 times to homogenize then discard the pipette tip. The microcentrifuge tube labeled “N1-100” now contains ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2) control at 10, 000 copies/mL.
4. Add 5 µL 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.

Note: The BioGX SARS-CoV-2 and Zeptometrix SARS-CoV-2 positive controls, when utilized as described (3,333 copies/mL) with the extracted workflow, may not sufficiently evaluate integrity of the assay reagents and could lead to false specimen results.

For further reference, please reference the product insert for **Lyophilized Control Template Beads (BioGX Product Number Series 720-0206)** which is available for download at www.biogx.com by using the drop down menu at the top right of the home page. Select "Education Center" then select "Product Documents". Choose the appropriate product number under "Template Controls".

Preparation of BioGX External Controls BioGX pixl.16

Positive Control [BioGX SARS-CoV-2 (N1)] - 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 to one well containing dispensed master mix.

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

All External Controls should yield the expected results (**Table 1**). An External Negative Control that yields a positive result is indicative of errant sample handling and/or contamination. An External Positive Control that yields a negative result is indicative of a specimen handling/preparation problem. Review the specimen handling/preparation technique.

An External Control that yields an Unresolved, Indeterminate, or Incomplete test result is indicative of a reagent or a real-time PCR system failure. Check the BioGX pixl.16 platform for any error messages. Refer to the "Troubleshooting" section of the BioGX pixl.16 Real-Time PCR¹² Instruction for Use Manual for interpretation of warning and error codes. If the problem persists, use reagents from an unopened pouch or use a new assay kit.

For further reference, please reference the product insert for **Lyophilized Control Template Beads (BioGX Product Number Series 720-0206)** which is available for download at www.biogx.com by using the drop down menu at the top right of the home page. Select "Education Center" then depending on your location select "Int. Product Documents" or "U.S. Product Documents". Choose the appropriate product number under "Template Controls".

Preparation of ZeptoMetrix External Positive Control for Extracted or Direct Samples

Preparation of ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2 Isolate: USA-WA1/2020) ZeptoMetrix catalog number: NATSARS(COV2)-ST

ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2) stock concentration of 1,000,000 copies/mL (1,000 copies/ μ L) should be serially diluted to achieve a final concentration of 10,000 copies/mL (10 copies/ μ L) for the extracted method:

1. With a fresh pipette tip, prepare **two** microcentrifuge tubes for serial dilution by adding 18 μ L of molecular grade water to each tube. Label the tube caps with “**N1-10**” and “**N1-100**” to represent the 10 times dilution and 100 times dilution of stock to yield 100 copies / μ L and 10 copies / μ L, respectively.
2. With a fresh pipette tip, transfer 2 μ L of the ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2) stock solution to the first microcentrifuge tube labeled “**ZM-10**”. Slowly pipette up and down 5 times to homogenize then discard the pipette tip.
3. With a fresh pipette tip, transfer 2 μ L of the “**N1-10**” dilution to the second microcentrifuge tube labeled “**N1-100**”. Slowly pipette up and down 5 times to homogenize then discard the pipette tip. The microcentrifuge tube labeled “**N1-100**” now contains ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2) control at 10,000 copies/mL.
4. 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.

Note: The BioGX SARS-CoV-2 and ZeptoMetrix SARS-CoV-2 positive controls, when utilized as described (3,333 copies/mL) with the extracted workflow, may not sufficiently evaluate integrity of the assay reagents and could lead to false specimen results.

RESULTS INTERPRETATION

Expected Results for Kit Controls

All test controls should be examined prior to interpretation of patient results. If the controls do not yield the expected results as shown in **Table 1** below, the patient results cannot be interpreted.

Table 1. Interpretation of BioGX external controls when run on **Applied Biosystems, Bio-Rad, BioGX pixl.16 or Bio Molecular Systems platforms.**

| Control Type | Applicability for Monitoring | N1 Gene | N1 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 | + | Ct≤30 |
| N1 Positive Control - BioGX Synthetic Template Control | Substantial reagent failure including primer and probe integrity | + | Ct≤40 | - | Not Detected | + | Ct≤30 |

An external negative control that yields a positive result for the COVID-19 N1 gene or RNase P gene is indicative of errant sample handling and/or contamination. If the N1 target or RNase P target is amplified, the assay should be repeated with reagents from an unopened pouch or new assay kit.

An external positive control that yields a negative result for the COVID-19 N1 target is indicative of a specimen handling and preparation and/or reagent problem. If N1 is not amplified, first review the specimen handling/preparation technique to ID handling/preparation errors then repeat the assay. If the issue persists, repeat with reagents from an unopened pouch or new assay kit.

The IAC, which is present in the RT-PCR master mix, should be detected and produce a Ct value ≤30 for all external negative and positive control reactions. If the IAC target is not detected, integrity of the assay components may be compromised, and the assay should be repeated with reagents from an unopened pouch or new assay kit.

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the external positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. The list of expected results is outlined in **Table 2**. If results are obtained that do not follow these guidelines, re-test sample or re-extract and re-test the sample if using extraction. If repeat testing yields similar results, collect a fresh sample from the patient for testing. In the presence of a high concentration positive result for the target, the IAC may or may not

amplify. This is normal. A positive test result does not necessarily indicate the presence of viable infectious organisms. A positive result is indicative of the presence of target nucleic acid. A negative test result does not preclude the presence of infectious organisms and should not be used as the sole basis for treatment or other patient management decisions.

Table 2. Interpretation of patient sample results¹⁶.

| Xfree™ COVID 19 Direct RT PCR Results | N1 gene | | RNase P | | IAC | | Result Interpretation |
|---|------------------|-----------------|------------------|-------------|------------------|----------------|------------------------------|
| | Detect (+ /) | Expected Ct | Detect (+ /) | Expected Ct | Detect (+ /) | Expected Ct | |
| SARS-CoV-2 POSITIVE | + | Ct≤40 | + | Ct≤36 | +/- | Ct≤30 | Report as POSITIVE |
| SARS-CoV-2 NEGATIVE | - | Not Detected | + | Ct≤36 | + | Ct≤30 | Report as NEGATIVE |
| Indeterminant | -/+ | Ct 0-40 | - / Ct≥36 | -/Ct≥36 | + | Ct≤30 | Repeat Test* |
| Indeterminant | -/+ | Ct 0-40 | + | Ct≤36 | - / Ct≥30 | - / Ct≥30 | Repeat Test* |

*Repeat the test by preparing a new test from the remaining patient sample collection and ensure the patient sample and PCR master mix is dispensed properly in the well being analyzed. Repeat testing must yield RNaseP and IAC detection without N1 detection to confirm a NEGATIVE result. However, if the same result is obtained upon a repeat test, report results as INCONCLUSIVE and request a new sample collection.

REPEAT TESTING FOR INSTRUMENT FAILURE

In case of instrument failure, repeat testing can be performed by setting up a new run using the original sample/specimen as described above in the “Specimen Preparation” section. Refer to the “Troubleshooting” section of the Applied Biosystems QuantStudio™ 5 (96-well and 384-well)⁹, Applied Biosystems 7500 Fast Dx¹⁰, Bio Rad CFX96 Touch™/CFX384 Touch™¹¹, BioGX pixl.16¹² and Bio Molecular Systems Mic¹³ Instruction Manuals for interpretation of warning and error codes.

LIMITATIONS OF THE PROCEDURE

- The use of this assay as an *in vitro* diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

- This product is intended for use with specimens collected using specimen collection and transport devices listed in the “Equipment and Materials Required But Not Provided” section.
- Incorrect test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up, or because the number of organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the BioGX Xfree™ COVID-19 assay instructions for use and the real-time PCR platforms (ie. Applied Biosystems QuantStudio™ 5 (96-well and 384-well)⁹, Applied Biosystems 7500 Fast Dx¹⁰, Bio-Rad CFX96 Touch™/CFX384 Touch™¹¹, BioGX pixl.16¹² and Bio Molecular Systems Mic¹³) are necessary to avoid erroneous results.
- Good laboratory technique is essential for the proper performance of this assay.
- The BioGX SARS-CoV-2 positive control, when utilized at 3,333 copies/mL with the extracted workflow, may not sufficiently evaluate integrity of the assay reagents and could lead to false specimen results.
- A positive test result does not necessarily indicate the presence of viable infectious organisms. A positive result is indicative of the presence of target nucleic acid. A negative test result does not preclude the presence of infectious organisms and should not be used as the sole basis for treatment or other patient management decisions.
- The BioGX Xfree™ COVID-19 assay results may sometimes be Unresolved or Indeterminate due to a lack of RNase P and/or IAC detection. Instrument failure could also contribute to an incomplete run result and require retesting that can lead to a delay obtaining final results.
- The performance of the BioGX Xfree™ COVID-19 assay was established using nasopharyngeal swab samples. Anterior nasal, mid-turbinate, and oropharyngeal swab specimens, and nasopharyngeal wash/aspirate or nasal aspirates are also considered acceptable specimen types for use with the BioGX Xfree™ COVID-19 assay but performance has not been established.
- Mutations or polymorphisms in primer- or probe-binding regions may affect detection of new or unknown SARS-CoV-2 variants resulting in a false negative result with the BioGX Xfree™ COVID-19 assay.
- The clinical performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The BioGX Xfree™ COVID-19 assay requires the use of three (3) optical channels on the Applied Biosystems QuantStudio™ 5 (96-well and 384-well), Applied

Biosystems 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™, BioGX pixl.16 and Bio Molecular Systems Mic platforms.

Conditions of Authorization for the Laboratory

The BioGX Xfree™ COVID-19 Direct RT-PCR Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on FDA website: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>.

However, to assist clinical laboratories using the BioGX Xfree™ COVID-19 Direct RT-PCR assay, the relevant Conditions of Authorization are listed below.

- a) Authorized laboratories¹ using the BioGX Xfree™ COVID-19 Direct RT-PCR assay must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- b) Authorized laboratories using the BioGX Xfree™ COVID-19 Direct RT-PCR assay must use the BioGX Xfree™ COVID-19 Direct RT-PCR assay as outlined in the authorized labeling. Deviations from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the BioGX Xfree™ COVID-19 Direct RT-PCR assay are not permitted.
- c) Authorized laboratories that receive the BioGX Xfree™ COVID-19 Direct RT-PCR assay must notify the relevant public health authorities of their intent to run the BioGX Xfree™ COVID-19 Direct RT-PCR assay prior to initiating testing.
- d) Authorized laboratories using the BioGX Xfree™ COVID-19 Direct RT-PCR assay must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- e) Authorized laboratories must collect information on the performance of the BioGX Xfree™ COVID-19 Direct RT-PCR assay and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRHEUA-Reporting@fda.hhs.gov) and BioGX, Inc. (via email: covid19@biogx.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the BioGX Xfree™ COVID-19 Direct RT-PCR assay of which they become aware.
- f) All laboratory personnel using the BioGX Xfree™ COVID-19 Direct RT-PCR assay must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the BioGX Xfree™ COVID-19 Direct RT-PCR assay in accordance with the authorized labeling.

g) BioGX, Inc. authorized distributors, and authorized laboratories using the BioGX Xfree™ COVID-19 Direct RT-PCR assay must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

1. The letter of authorization refers to, “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42U.S.C. §263a, that meet requirements to perform high complexity tests” as “authorized laboratories.”

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity - Direct Patient Sample

Limit of Detection (LoD) was determined by serially diluting quantified inactivated SARS-CoV-2 (USA-WA1/2020; Zeptomatrix catalog number NATSARS(CoV2)-ST) into negative NP swabs samples in Copan UTM®/VTM, Saline, and Copan ESwab™ transport media. The LoD for contrived samples in saline, Copan ESwab™, or Copan UTM®/VTM was then confirmed by testing additional replicates (n=20) of inactivated SARS-CoV-2 virus. LoD confirmation was performed using the following real-time PCR platforms: Applied Biosystems QuantStudio™ 5 (96-well and 384-well), Applied Biosystems 7500 Fast Dx, Bio-Rad CFX96 Touch™ and Bio-Rad CFX384 Touch™, BioGX pixl.16 and Bio Molecular Systems Mic. The LoD for the assay using direct patient samples on the validated platforms ranged from 1,000 – 3,000 copies/mL (**Table 3**).

Table 3. LoD confirmation -Direct Patient Sample

| Instrument | Transport Media | Concentration [Copies/ mL] | N1 gene | | RNase P | IAC |
|---------------------------------|-----------------|----------------------------|---|---------|---------|---------|
| | | | Agreement with Expected Result (% Positive) | Mean Ct | Mean Ct | Mean Ct |
| QuantStudio™ 5 (0.2 mL 96-well) | UTM®/VTM | 1000 | 19/20 (95%) | 35.1 | 30.0 | 24.1 |
| | Saline | 1000 | 19/20 (95%) | 34.2 | 31.4 | 24.4 |
| | Eswab™ | 3000 | 20/20 (100%) | 34.1 | 29.2 | 25.4 |
| QuantStudio™ 5 (384-well) | UTM®/VTM | 2000 | 20/20 (100%) | 34.6 | 31.8 | 23.1 |
| | Saline | 2500 | 19/20 (95%) | 34.8 | 28.1 | 19.0 |
| | Eswab™ | 3000 | 20/20 (100%) | 32.9 | 31.9 | 16.3 |
| 7500 Fast Dx | UTM®/VTM | 1000 | 20/20 (100%) | 34.6 | 30.6 | 26.4 |
| | Saline | 1000 | 20/20 (100%) | 35.3 | 30.8 | 26.2 |
| | Eswab™ | 2000 | 20/20 (100%) | 34.6 | 28.8 | 28.1 |
| CFX96 Touch™ | UTM®/VTM | 1500 | 19/20 (95%) | 36.4 | 30.4 | 27.4 |
| | Saline | 1500 | 20/20 (100%) | 35.6 | 33.2 | 27.9 |
| | Eswab™ | 1500 | 19/20 (95%) | 35.9 | 30.1 | 28.3 |
| CFX384 Touch™ | UTM®/VTM | 1000 | 19/20 (95%) | 36.3 | 31.2 | 27.2 |

| | | | | | | |
|------------------------------|----------|------|--------------|------|------|------|
| | Saline | 2000 | 20/20 (100%) | 36.6 | 32.9 | 27.5 |
| | Eswab™ | 3000 | 20/20 (100%) | 36.1 | 28.6 | 29.0 |
| BioGX pixl.16 | UTM®/VTM | 2000 | 19/20 (95%) | 31.1 | 31.9 | 25.6 |
| | Saline | 3000 | 19/20 (95%) | 32.1 | 29.3 | 25.2 |
| Bio Molecular Systems Mic | UTM®/VTM | 1000 | 21/22 (96%) | 34.0 | 29.2 | 26.8 |
| | Saline | 1500 | 22/22 (100%) | 33.6 | 31.2 | 26.4 |
| | Eswab™ | 2000 | 22/22 (100%) | 33.9 | 29.1 | 27.3 |

Analytical Sensitivity – Extracted Patient Sample

Limit of Detection (LoD) was determined by serially diluting quantified inactivated SARS-CoV-2 (USA-WA1/2020; Zeptomatrix catalog number NATSARS(CoV2)-ST) into negative NP swab samples in saline, Copan Eswab™, or Copan UTM®/VTM, which were then extracted with the Qiagen QIAamp Viral RNA Mini Kit as described above. The LoD for contrived samples in saline, Copan Eswab™, or Copan UTM®/VTM was then confirmed by testing additional replicates (n=20) of inactivated SARS-CoV-2 virus. LoD confirmation was performed using the following real-time PCR platforms: Applied Biosystems QuantStudio™ 5 (96-well and 384-well), Applied Biosystems 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™, BioGX pixl.16 and Bio Molecular Systems Mic. The LoD for the assay using extracted patient samples on the validated platforms ranged from 125 - 500 copies/mL (**Table 4**).

Table 4. LoD confirmation – Extracted Patient Sample

| Instrument | Transport Media | Concentration [Copies/ mL] | N1 gene | | RNase P | IAC |
|---------------------------------|-----------------|----------------------------|---|---------|---------|---------|
| | | | Agreement with Expected Result (% Positive) | Mean Ct | Mean Ct | Mean Ct |
| QuantStudio™ 5 (0.2 mL 96-well) | UTM®/VTM | 250 | 20/20 (100%) | 33.9 | 25.5 | 23.5 |
| | Saline | 250 | 20/20 (100%) | 34.6 | 29.3 | 23.5 |
| | ESwab™ | 500 | 19/20 (95%) | 35.2 | 28.2 | 23.5 |
| QuantStudio™ 5 (384-well) | UTM®/VTM | 250 | 19/20 (95%) | 32.0 | 26.4 | 15.8 |
| | Saline | 250 | 19/20 (95%) | 34.8 | 27.8 | 17.4 |
| | ESwab™ | 500 | 19/20 (95%) | 34.7 | 27.9 | 23.9 |
| 7500 Fast Dx | UTM®/VTM | 125 | 19/20 (95%) | 35.2 | 28.4 | 26.1 |
| | Saline | 250 | 19/20 (95%) | 34.7 | 29.2 | 26.1 |
| | ESwab™ | 250 | 19/20 (95%) | 35.3 | 27.1 | 26.0 |
| CFX96 Touch™ | UTM®/VTM | 250 | 20/20 (100%) | 35.8 | 25.7 | 26.7 |
| | Saline | 250 | 20/20 (100%) | 36.0 | 29.5 | 26.9 |
| | ESwab™ | 250 | 19/20 (95%) | 36.3 | 27.1 | 26.8 |
| CFX384 Touch™ | UTM®/VTM | 500 | 20/20 (200%) | 36.7 | 26.9 | 26.5 |
| | Saline | 500 | 20/20 (100%) | 36.5 | 29.6 | 26.4 |
| | ESwab™ | 500 | 20/20 (100%) | 36.9 | 29.5 | 26.6 |
| BioGX pixl.16 | UTM®/VTM | 250 | 20/20 (100%) | 33.3 | 29.7 | 24.8 |
| | Saline | 500 | 20/20 (100%) | 32.5 | 27.6 | 25.0 |
| Bio Molecular Systems Mic | UTM®/VTM | 750 | 22/22 (100%) | 33.6 | 27.3 | 25.3 |
| | Saline | 750 | 22/22 (100%) | 33.6 | 29.3 | 25.2 |
| | ESwab™ | 500 | 22/22 (100%) | 33.5 | 28.6 | 26.2 |

*Qiagen Viral RNA Mini extraction kit used according to manufacturer recommendations (i.e. 140µL sample processed and eluted in 50µL).

Inclusivity (*in-silico*)

The Xfree™ COVID-19 assay primer and probe sequences are identical to the FDA EUA authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. CDC conducted the *in silico* inclusivity analysis. The data from this analysis is available in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC) Instructions For Use document, to which the CDC has granted a broad right of reference.

An additional *in silico* analysis assessing the impact of the recently emerged SARS-CoV-2 variants on target detection by BioGX showed that none of the observed mutations would be expected to interfere with N gene detection.

Cross-Reactivity (Analytical Specificity)

The Xfree™ COVID-19 assay primer and probe sequences are identical to the FDA EUA authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. CDC conducted the *in silico* cross-reactivity analysis. The data from this analysis is available in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC) Instructions For Use document, to which the CDC has granted a broad right of reference.

Interfering Substances

An interfering substances study was conducted to evaluate the potential of endogenous substances to cause both false positive and false negative results with the Xfree™ COVID-19 assay when using the direct workflow.

For this study, potential interfering substances were spiked into pooled Copan UTM® negative nasopharyngeal swab samples containing no viral RNA or SARS-CoV-2 (USA-WA1/2020; Zeptomatrix catalog number NATSARS(CoV2)-ST) at approximately 5X LoD (5,000 copies/mL). **Table 6** summarizes the results obtained from negative (SARS-CoV-2 Negative NP) and positive (SARS-CoV-2 Positive NP) replicate runs (n=3) that were conducted for each substance. All runs were conducted following the direct sample workflow and analyzed with both the Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well) and Bio-Rad CFX96 Touch™. All samples yielded the expected results, indicating an absence of interference with the tested substances.

Table 6. Analysis of interfering substances.

| Potential Interfering substance | Final concentration (in sample) | Agreement with expected results | | | |
|---|---------------------------------|---------------------------------|------------------------|------------------------|------------------------|
| | | QuantStudio™ 5 (0.2 mL 96 well) | | CFX96 Touch™ | |
| | | SARS CoV 2 Positive NP | SARS CoV 2 Negative NP | SARS CoV 2 Positive NP | SARS CoV 2 Negative NP |
| None | N/A | 100% | 100% | 100% | 100% |
| Mucin (bovine submaxillary gland, type I-S) | 2.5 mg/mL | 100% | 100% | 100% | 100% |

| | | | | | |
|---|--------------------------------------|------|------|------|------|
| Mucin (bovine submaxillary gland, type I-S) | 1.25 mg/mL | 100% | 100% | 100% | 100% |
| Nasacort | 10% v/v | 100% | 100% | 100% | 100% |
| NeilMed NasoGEL | 1.25% w/v | 100% | 100% | 100% | 100% |
| Similasan Nasal Allergy Relief™ | 15% v/v | 100% | 100% | 100% | 100% |
| Saline Nasal Spray | 15% v/v | 100% | 100% | 100% | 100% |
| Act dry mouth lozenges | 0.04 g/mL | 100% | 100% | 100% | 100% |
| Chloraseptic® Spray | 10% v/v | 100% | 100% | 100% | 100% |
| Cepacol Extra Strength Cherry Lozenges | 0.046 g/mL (0.15mg/mL Benzocaine) | 100% | 100% | 100% | 100% |
| Chloraseptic Lozenges | 0.071 g/mL (0.12mg/mL Benzocaine) | 100% | 100% | 100% | 100% |
| Halls Cough drops | 0.062 g/mL (0.15 mg/mL Menthol) | 100% | 100% | 100% | 100% |
| Nature's Bounty® Zinc (50mg) | 10µg/mL | 100% | 100% | 100% | 100% |
| Nyquil | 10% v/v | 100% | 100% | 100% | 100% |
| Robitussin | 10% v/v | 100% | 100% | 100% | 100% |

| | | | | | |
|--|-----------|------|------|------|------|
| Flonase | 5% v/v | 100% | 100% | 100% | 100% |
| Ethanol | 5% v/v | 100% | 100% | 100% | 100% |
| ORIGINAL LISTERINE® Mouthwash | 5% v/v | 100% | 100% | 100% | 100% |
| Colgate Toothpaste Original | 1% v/v | 100% | 100% | 100% | 100% |
| Mupirocin Ointment (Bactroban) | 5 mg/mL | 100% | 100% | 100% | 100% |
| Tobramycin (Antibiotic) | 100 ng/mL | 100% | 100% | 100% | 100% |
| Oseltamivir (Tamiflu API) | 3.3 mg/mL | 100% | 100% | 100% | 100% |
| Emergen-C Vitamin C Drink - Super Orange | 10% v/v | 100% | 100% | 100% | 100% |
| Petroleum jelly (Vaseline) | 1% w/v | 100% | 100% | 100% | 100% |
| Tobacco | 1% v/v | 100% | 100% | 100% | 100% |

Clinical Performance

Positive percent agreement (PPA) and negative percent agreement (NPA) between the BioGX Xfree™ COVID-19 assay and a highly sensitive FDA EUA authorized RT-PCR comparator was determined using 60 individual SARS-CoV-2 positive and 35 individual SARS-CoV-2 negative clinical nasopharyngeal swab samples collected in UTM/VTM and collected by qualified healthcare professionals from individuals suspected of SARS-CoV-2 infection. Samples were handled as described in the package insert of the collection device and stored frozen until use.

Testing with Direct Patient Sample Addition

The clinical samples were tested by direct sample addition (5 µL of clinical sample transferred directly into 15 µL of the BioGX Xfree™ COVID-19 mastermix). Clinical samples were analyzed on the QuantStudio™ 5 (0.2 mL 96-well) following the device instructions for use. The BioGX Xfree™ COVID-19 assay, when using the direct sample addition workflow achieved a PPA of 95% and an NPA of 100% when compared to the comparator method (**Table 7**).

Testing with Extracted Patient Samples

The clinical samples were also tested following the extracted patient sample workflow with the Qiagen QIAamp Viral RNA Mini Kit following the manufacturer’s recommendations, where 140 µL of clinical sample were extracted and then eluted with 50 µL RNase-free water. The eluted nucleic acid template (5 µL) was added to 10 µL of the BioGX Xfree™ master mix. Positive clinical patient samples (n=60) and negative clinical patient samples (n=35) yielded a PPA and NPA of 100% and 94.29%, respectively, when analyzed on the QuantStudio™ 5 (0.2 mL 96-well) (**Table 8**).

Table 7. Clinical performance of the BioGX Xfree™ COVID-19 direct sample addition workflow

| BioGX Xfree™ COVID 19 Assay (Direct Sample Addition) | Comparator RT PCR EUA Test | |
|---|-------------------------------|----------|
| | Positive | Negative |
| Positive | 57 | 0 |
| Negative | 3* | 35 |
| Positive Percent Agreement (95%CI) | 95.0% (86.3-98.3%) | |
| Negative Percent Agreement (95%CI) | 100.0% (90.1-100.0%) | |

*The three direct samples with discordant results were identified as positive by the comparator method with Ct values between 37.6 & 40.5.

Table 8. Clinical performance of the BioGX Xfree™ COVID-19 extracted sample workflow

| BioGX Xfree™ COVID 19 Assay (Extracted Sample Workflow) | Comparator RT PCR EUA Test | |
|--|-------------------------------|-----------------|
| | <i>Positive</i> | <i>Negative</i> |
| <i>Positive</i> | 60 | 2* |
| <i>Negative</i> | 0 | 33 |
| <i>Positive Percent Agreement</i> | 100.0% (94.0-100.0%) | |
| <i>Negative Percent Agreement</i> | 94.3% (81.4-98.4%) | |

*The two samples that tested positive with the BioGX Xfree™ COVID-19 assay and negative with the comparator method was tested with a third, highly sensitive RT-PCR EUA authorized test. Both samples tested positive with the second RT-PCR EUA authorized test.

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APPENDIX A: Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well) Programming

Direct Patient Sample (20 µL reaction volume)

Please refer to the following steps for **QuantStudio™ 5** manual programming specific for Direct Patient sample testing.

1. Open the QuantStudio Design and Analysis Software v1.5.1
2. Select the “Create New Experiment” option
3. Within the properties tab, select the following properties to filter:
 - a. Name: User defined or 500003XMPDirectQS5v2
 - b. Instrument Type: QuantStudio 5 (0.2 mL 96-well) System
 - c. Block Type: 96-Well 0.2 mL
 - d. Experiment type: Standard Curve
 - e. Chemistry: TaqMan Reagents
 - f. Run mode: Standard
4. Run Method (Method Tab)
 - a. Change the Reaction Volume to **20.0 µL**
 - b. Temperature of the enabled heated cover will remain at 105.0 °C
 - c. Within the “PCR Stage “ section of the thermocycling parameters, an additional step will need to be added by bringing the mouse pointer above “Step 2” will allow the “+” and “-” buttons to become visible.
 - d. Click the “+” button to add “Step 3” to the “PCR Stage”.
 - e. Going back to the first hold stage enter the following parameters (**Table A1**):
 - i. Hold Stage: Step 1
 1. 1.6 ramp rate
 2. 46°C
 3. 20 minutes
 - ii. Hold Stage: Step 2
 1. 1.6 ramp rate
 2. 95°C
 3. 5 minutes
 - iii. PCR Stage: Step 1
 1. 1.6 ramp rate
 2. 95°C
 3. 3 seconds

- iv. PCR Stage: Step 2
 - 1. 1.6 ramp rate
 - 2. 62°C
 - 3. 20 seconds
 - 4. Scanning is turned ON by clicking the camera icon (dark blue when ON).
- v. PCR Stage: Step 3
 - 1. 1.6 ramp rate
 - 2. 70°C
 - 3. 5 seconds
 - 4. Scanning is turned OFF by clicking the camera icon (light blue when OFF).
- vi. Number of cycles set to 40 (located at the bottom of the “PCR Stage” section).

Table A1. QuantStudio™ 5 (0.2 mL 96-well) thermocycling summary - Direct Patient Sample.

| PCR Cycle Summary | | | | | |
|--------------------|------------|--------|-----------|--------|--------|
| | Hold Stage | | PCR Stage | | |
| Conditions | Step 1 | Step 2 | Step 1 | Step 2 | Step 3 |
| Ramp Rate (°C/s) | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 |
| Temperature (°C/s) | 46 | 95 | 95 | 62 | 70 |
| Duration (Min:sec) | 20:00 | 5:00 | 0:03 | 0:20 | 0:05 |
| Detection | OFF | OFF | OFF | ON | OFF |
| No. of PCR Cycles | HOLD | HOLD | 40 | | |

5. Plate Setup (Plate Tab)

- a. Under the “Plate Attribute” section of the Quick Setup tab, change the Passive Reference to “NONE”.
- b. On the left side of the screen (next to Quick Setup), choose “Advanced Setup”.
- c. In the “Targets” section, click on the “Add” box and add two targets for a total of three. Rename Target1 as “RNaseP”.

- d. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “FAM”.
- e. Click on the “Target 2” box and change the name to “N1”.
- f. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “ROX”.
- g. Click on the “Target 3” box and change the name to “IAC”.
- h. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “Cy5”.
- i. Ensure that the “Quencher” tab for all three targets is set to “None”.
- j. Highlight all the wells that you intend to use and ensure that the boxes besides the three targets are selected and in the task select “U” for unknown. This should populate the plate on the right side of the screen with each target.
- k. To name your wells by sample name, select the appropriate well on the right side of the screen. Under the “Samples” tab on the left side, click on the current sample name and rename to whatever is desired for the experiment. Check the box besides the sample name: the names will populate on the right side.
- l. To add additional sample names, click “Add” on the samples tab and repeat the instructions above.
- m. Click on the “Results” tab on the top of the screen

6. Threshold Setup (Results Tab)

- a. Navigate to the “Results” tab and select the settings wheel besides “Analyze” in the upper right corner.
- b. Click on target “N1” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 10,000.**
- c. Click on target “RNaseP” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 25,000.**
- d. Click on target “IAC” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 5,000.**
- e. Click “Apply” in the bottom right corner.

7. Save File

- a. Navigate to the “File” tab and choose “Save As”. Name the file ‘500003XMPDirectQS5v2’ and ensure it is saved as an *.edt file.

8. Run File

- a. Select “File” then “Open”.
- b. Select your chosen *.edt file.
- c. Navigate to the “Run” tab, and click the dropdown arrow next to “Start Run”. Ensure the name of the instrument is your preferred instrument. You will be prompted to enter a name for the run.

- d. Insert the desired run name and click “Start Run”.

Applied Biosystems QuantStudio™ 5 (0.2 mL 96-well) Programming

Extracted Patient Sample (15 µL reaction volume)

Please refer to the following steps for **QuantStudio™ 5** manual programming specific for Extracted Patient sample testing.

1. Open the QuantStudio Design and Analysis Software v1.5.1
2. Select the “Create New Experiment” option
3. Within the properties tab, select the following properties to filter:
 - a. Name: User defined or 500003XMPExtractQS5v2
 - b. Instrument Type: QuantStudio 5 System (0.2 mL 96-well)
 - c. Block Type: 96-Well 0.2 mL
 - d. Experiment type: Standard Curve
 - e. Chemistry: TaqMan Reagents
 - f. Run mode: Standard
4. Run Method (Method Tab)
 - a. Change the Reaction Volume to **15.0 µL**
 - b. Temperature of the enabled heated cover will remain at 105.0 °C
 - c. Within the “PCR Stage “ section of the thermocycling parameters, an additional step will need to be added by bringing the mouse pointer above “Step 2” will allow the “+” and “-” buttons to become visible.
 - d. Click the “+” button to add “Step 3” to the “PCR Stage”.
 - e. Going back to the first hold stage enter the following parameters (**Table A1**):
 - i. Hold Stage: Step 1
 1. 1.6 ramp rate
 2. 46°C
 3. 20 minutes
 - ii. Hold Stage: Step 2
 1. 1.6 ramp rate
 2. 95°C
 3. 5 minutes
 - iii. PCR Stage: Step 1
 1. 1.6 ramp rate
 2. 95°C
 3. 3 seconds
 - iv. PCR Stage: Step 2
 1. 1.6 ramp rate
 2. 62°C
 3. 20 seconds
 4. Scanning is turned ON by clicking the camera icon (dark blue when ON).
 - v. PCR Stage: Step 3

1. 1.6 ramp rate
 2. 70°C
 3. 5 seconds
 4. Scanning is turned OFF by clicking the camera icon (light blue when OFF).
- vi. Ensure the number of cycles is set to 40 (located at the bottom border of the PCR Stage).

5. Plate Setup (Plate Tab)

- a. Under the “Plate Attribute” section of the Quick Setup tab, change the Passive Reference to “NONE”.
- b. On the left side of the screen (next to Quick Setup), choose “Advanced Setup”.
- c. In the “Targets” section, click on the “Add” box and add two targets for a total of three. Rename Target1 as “RNaseP”.
- d. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “FAM”.
- e. Click on the “Target 2” box and change the name to “N1”.
- f. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “ROX”.
- g. Click on the “Target 3” box and change the name to “IAC”.
- h. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “Cy5”
- i. Ensure that the “Quencher” tab for all three targets is set to “None”.
- j. Highlight all the wells that you intend to use and ensure that the boxes besides the three targets are selected. In the “Task” section select “U” for unknown. This should populate the plate on the right side of the screen with each target.
- k. To name your wells by sample name, select the appropriate well on the right side of the screen. Under the “Samples” tab on the left side, click on the current sample name and rename to whatever is desired for the experiment. Check the box besides the sample name: the names will populate on the right side.
- l. To add additional sample names, click “Add” on the samples tab and repeat the instructions above.
- m. Click on the “Results” tab on the top of the screen

6. Threshold Setup (Results Tab)

- a. Navigate to the “Results” tab and select the settings wheel besides “Analyze” in the upper right corner.
- b. Click on target “N1” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 10,000.**
- c. Click on target “RNaseP” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 25,000.**
- d. Click on target “IAC” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 5,000.**
- e. Click “Apply” in the bottom right corner.

7. Save File

- a. Navigate to the “File” tab and choose “Save As”. Name the file ‘500003XMPExtractQS5v2’ and ensure it is saved as an *.edt file.

8. Run File

- a. Select “File” then “Open”.
- b. Select your chosen *.edt file.
- c. Navigate to the “Run” tab, and click the dropdown arrow next to “Start Run”. Ensure the name of the instrument is your preferred instrument. You will be prompted to enter a name for the run.
- d. Insert the desired run name and click “Start Run”.

APPENDIX B: Applied Biosystems QuantStudio™ 5 (384-well) Programing

Direct Patient Sample (20 µL reaction volume)

Please refer to the following steps for **QuantStudio™ 5 (384-well)** manual programming specific for Direct Patient sample testing.

1. Open the QuantStudio Design and Analysis Software v1.5.1
2. Select the “Create New Experiment” option
3. Within the properties tab, select the following properties to filter:
 - 1) Name: User defined or 500003XMPDirectQS5_384Rev01.edt
 - 2) Instrument Type: QuantStudio 5, 384-well System
 - 3) Block Type: 384-Well 0.2 mL
 - 4) Experiment type: Standard Curve
 - 5) Chemistry: TaqMan Reagents
 - 6) Run mode: Standard
4. Run Method (Method Tab)
 - a. Change the Reaction Volume to **20.0 µL**
 - b. Temperature of the enabled heated cover will remain at 105.0°C
 - c. Within the “PCR Stage “ section of the thermocycling parameters, an additional step will need to be added by bringing the mouse pointer above “Step 2” will allow the “+” and “-” buttons to become visible.
 - d. Click the “+” button to add “Step 3” to the “PCR Stage”.
 - e. Going back to the first hold stage enter the following parameters (**Table B1**):
 - i. Hold Stage: Step 1
 1. 1.6 ramp rate
 2. 46°C
 3. 20 minutes
 - ii. Hold Stage: Step 2
 1. 1.6 ramp rate
 2. 95°C
 3. 5 minutes
 - iii. PCR Stage: Step 1
 1. 1.6 ramp rate
 2. 95°C
 3. 3 seconds

- iv. PCR Stage: Step 2
 - 1. 1.6 ramp rate
 - 2. 62°C
 - 3. 20 seconds
 - 4. Scanning is turned ON by clicking the camera icon (dark blue when ON).
- v. PCR Stage: Step 3
 - 1. 1.6 ramp rate
 - 2. 70°C
 - 3. 5 seconds
 - 4. Scanning is turned OFF by clicking the camera icon (light blue when OFF).
- vi. Number of cycles set to 40 (located at the bottom of the “PCR Stage” section).

Table B1. QuantStudio™ 5 (384-well) thermocycling summary - Direct Patient Sample.

| PCR Cycle Summary | | | | | |
|--------------------|------------|--------|-----------|--------|--------|
| | Hold Stage | | PCR Stage | | |
| Conditions | Step 1 | Step 2 | Step 1 | Step 2 | Step 3 |
| Ramp Rate (°C/s) | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 |
| Temperature (°C) | 46 | 95 | 95 | 62 | 70 |
| Duration (Min:sec) | 20:00 | 5:00 | 0:03 | 0:20 | 0:05 |
| Detection | OFF | OFF | OFF | ON | OFF |
| No. of PCR Cycles | 1 | 1 | 40 | | |

5. Plate Setup (Plate Tab)

- a. Under the “Plate Attribute” section of the Quick Setup tab, change the Passive Reference to “NONE”.
- b. On the left side of the screen (next to Quick Setup), choose “Advanced Setup”.
- c. In the “Targets” section, click on the “Add” box and add two targets for a total of three. Rename “Target 1” as “RNaseP”.
- d. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “FAM”.
- e. Click on the “Target 2” box and change the name to “N1”.

- f. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “ROX”.
- g. Click on the “Target 3” box and change the name to “IAC”.
- h. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “Cy5”
- i. Ensure that the “Quencher” tab for all three targets is set to “None”.
- j. Highlight all the wells that you intend to use and ensure that the boxes besides the three targets are selected and in the task select “U” for unknown. This should populate the plate on the right side of the screen with each target.
- k. To name your wells by sample name, select the appropriate well on the right side of the screen. Under the “Samples” tab on the left side, click on the current sample name and rename to whatever is desired for the experiment. Check the box besides the sample name: the names will populate on the right side.
- l. To add additional sample names, click “Add” on the samples tab and repeat the instructions above.
- m. Click on the “Results” tab on the top of the screen

6. Threshold Setup (Results Tab)

- a. Navigate to the “Results” tab and select the settings wheel besides “Analyze” in the upper right corner.
- b. Click on target “N1” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 10,000.**
- c. Click on target “RNaseP” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 25,000.**
- d. Click on target “IAC” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 5,000.**
- e. Click “Apply” in the bottom right corner.

7. Save File

- a. Navigate to the “File” tab and choose “Save As”. Name the file ‘500003XMPDirectQS5_384Rev01.edt’ and ensure it is saved as an *.edt file.

8. Run File

- a. Select “File” then “Open”.
- b. Select your chosen *.edt file.
- c. Navigate to the “Run” tab, and click the dropdown arrow next to “Start Run”. Ensure the name of the instrument is your preferred instrument. You will be prompted to enter a name for the run.
- d. Insert the desired run name and click “Start Run”.

Applied Biosystems QuantStudio™ 5 (384-well) Programming

Extracted Patient Sample (15 µL reaction volume)

Please refer to the following steps for **QuantStudio™ 5 (384-well)** manual programming specific for Extracted Patient sample testing.

1. Open the QuantStudio Design and Analysis Software v1.5.1
2. Select the “Create New Experiment” option
3. Within the properties tab, select the following properties to filter:
 - a. Name: User defined or 500003XMPExtractedQS5_384Rev01.edt
 - b. Instrument Type: QuantStudio 5, 384-well System
 - c. Block Type: 384-Well 0.2 mL
 - d. Experiment type: Standard Curve
 - e. Chemistry: TaqMan Reagents
 - f. Run mode: Standard
4. Run Method (Method Tab)
 - a. Change the Reaction Volume to **15.0 µL**
 - b. Temperature of the enabled heated cover will remain at 105.0°C
 - c. Within the “PCR Stage “ section of the thermocycling parameters, an additional step will need to be added by bringing the mouse pointer above “Step 2” will allow the “+” and “-” buttons to become visible.
 - d. Click the “+” button to add “Step 3” to the “PCR Stage”.
 - e. Going back to the first hold stage enter the following parameters (**Table B1**):
 - i. Hold Stage: Step 1
 1. 1.6 ramp rate
 2. 46°C
 3. 20 minutes
 - ii. Hold Stage: Step 2
 1. 1.6 ramp rate
 2. 95°C
 3. 5 minutes
 - iii. PCR Stage: Step 1
 1. 1.6 ramp rate
 2. 95°C
 3. 3 seconds
 - iv. PCR Stage: Step 2

1. 1.6 ramp rate
 2. 62°C
 3. 20 seconds
 4. Scanning is turned ON by clicking the camera icon (dark blue when ON).
 - v. PCR Stage: Step 3
 1. 1.6 ramp rate
 2. 70°C
 3. 5 seconds
 4. Scanning is turned OFF by clicking the camera icon (light blue when OFF).
 - vi. Ensure the number of cycles is set to 40 (located at the bottom border of the PCR Stage).
5. Plate Setup (Plate Tab)
 - a. Under the “Plate Attribute” section of the Quick Setup tab, change the Passive Reference to “NONE”.
 - b. On the left side of the screen (next to Quick Setup), choose “Advanced Setup”.
 - c. In the “Targets” section, click on the “Add” box and add two targets for a total of three. Rename “Target 1” as “RNaseP”.
 - d. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “FAM”.
 - e. Click on the “Target 2” box and change the name to “N1”.
 - f. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “ROX”.
 - g. Click on the “Target 3” box and change the name to “IAC”.
 - h. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose “Cy5”
 - i. Ensure that the “Quencher” tab for all three targets is set to “None”.
 - j. Highlight all the wells that you intend to use and ensure that the boxes besides the three targets are selected. In the “Task” section select “U” for unknown. This should populate the plate on the right side of the screen with each target.
 - k. To name your wells by sample name, select the appropriate well on the right side of the screen. Under the “Samples” tab on the left side, click on the current sample name and rename to whatever is desired for the experiment. Check the box besides the sample name: the names will populate on the right side.
 - l. To add additional sample names, click “Add” on the samples tab and repeat the instructions above.
 - m. Click on the “Results” tab on the top of the screen
6. Threshold Setup (Results Tab)
 - a. Navigate to the “Results” tab and select the settings wheel besides “Analyze” in the upper right corner.

- b. Click on target “N1” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 10,000.**
- c. Click on target “RNaseP” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 25,000.**
- d. Click on target “IAC” and uncheck the “Ct Settings to Use: Default Settings” box directly to the right. Then uncheck the “Automatic Threshold” box: **set the threshold to 5,000.**
- e. Click “Apply” in the bottom right corner.

7. Save File

- a. Navigate to the “File” tab and choose “Save As”. Name the file ‘500003XMPExtractedQS5_384Rev01.edt’ and ensure it is saved as an *.edt file.

8. Run File

- a. Select “File” then “Open”.
- b. Select your chosen *.edt file.
- c. Navigate to the “Run” tab, and click the dropdown arrow next to “Start Run”. Ensure the name of the instrument is your preferred instrument. You will be prompted to enter a name for the run.
- d. Insert the desired run name and click “Start Run”.

APPENDIX C: Applied Biosystems 7500 Fast Dx Programming

Direct Patient Sample (20 µL reaction volume)

Please refer to the following steps for Applied Biosystems 7500 Fast Dx manual programming specific for Direct Patient sample testing.

1. Open the Applied Biosystems 7500 Fast Dx software v1.4.1
2. Go to the “Tools” tab and navigate to “Detector Manager”
 - a. Click on the “File” dropdown and select “New”.
 - b. Name the target as “N1”
 - i. Set the reporter dye to “ROX”
 - ii. Set the quencher dye to none
 - iii. Click Apply
 - c. Name the target as “RNaseP”
 - i. Set the reporter dye to “FAM”
 - ii. Set the quencher dye to “None”
 - iii. Click Apply
 - d. Name the target as “IAC”
 - i. Set the reporter dye to “Cy5”
 - ii. Set the quencher dye to “None”
 - iii. Click Apply
3. Click the “File” tab and select “New”
4. On the “Define document” Pop-Up:
 - a. Assay: Standard Curve (Absolute Quantification)
 - b. Container: 96 Well Clear
 - c. Template: Blank Document
 - d. Run Mode: Fast 7500
 - e. Operator: Select the operator you wish to run the assay with
 - f. Plate Name: Select your desired name for the experiment file
 - g. Click “Next”
5. Select the previously created detectors from the library: N1, RNaseP, IAC and click add.
6. Set the Passive Reference to “None”
7. Click “next”. Select all the wells and assign the targets to them.
8. Select Finish.
9. Navigate to the Instrument tab.
 - a. Change the Sample Volume to **20.0 µL**
 - b. Click the “Add Hold” button until you have two hold steps. Set the number of repetitions to 1.
 - c. Click the “Add Step” button until you have three PCR cycling steps. Set the number of repetitions for this stage to 40.
 - d. Going back to the first hold stage enter, the following parameters (**Table C1**):
 - i. Hold Stage: Step 1
 1. Auto ramp rate
 2. 46°C
 3. 20 minutes
 - ii. Hold Stage: Step 2

1. Auto ramp rate
 2. 95°C
 3. 5 minutes
 - iii. PCR Stage: Step 1
 1. Auto ramp rate
 2. 95°C
 3. 3 seconds
 - iv. PCR Stage: Step 2
 1. Auto ramp rate
 2. 62°C
 3. 24 seconds.
 - v. PCR Stage: Step 3
 1. Auto ramp rate
 2. 70°C
 3. 5 seconds
 - vi. Under settings, select the following:
 1. Sample Volume: 20
 2. Run Mode: Fast 7500
 3. Data Calculation: Stage 3, step 2
- e. Select the “Analysis” tab and click “Analysis settings”.
 - i. Select N1’s target settings by clicking on the detector dropdown.
 1. Select “Manual CT” and **set the threshold to 50,000**
 2. Select “Manual Baseline” and set the start cycle to 3 and the end to 10.
 3. Click apply
 - ii. Select RNaseP
 1. Select Manual CT and **set the threshold to 100,000**
 2. Select “Manual Baseline” and set the start cycle to 3 and the end to 15
 3. Click apply
 - iii. Select IAC
 1. Select Manual CT and **set the threshold to 100,000**
 2. Select “Manual Baseline” and set the start cycle to 3 and the end to 20
 3. Click “Apply”, then select “OK”.
- f. Select File -> Save. Set your desired name and your template is saved.

Table C1. Applied Biosystems 7500 thermocycling summary - Direct Patient Sample.

| PCR Cycle Summary | | | | | |
|--------------------|------------|--------|-----------|-----------|--------|
| | Hold Stage | | PCR Stage | | |
| Conditions | Step 1 | Step 2 | Step 1 | Step 2 | Step 3 |
| Ramp Rate (°C/s) | Auto | Auto | Auto | Auto | Auto |
| Temperature (°C/s) | 46 | 95 | 95 | 62 | 70 |
| Duration (Min:sec) | 20:00 | 5:00 | 0:03 | 0:24 | 0:05 |
| Detection | OFF | OFF | OFF | ON | OFF |
| No. of PCR Cycles | HOLD | HOLD | 40 | | |

Applied Biosystems 7500 Fast Dx Programming

Extracted Patient Sample (15 µL reaction volume)

Please refer to the following steps for **Applied Biosystems 7500 Fast Dx** manual programming specific for Extracted patient sample testing.

1. Open the Applied Biosystems 7500 Fast Dx software v1.4.1
2. Go to the “Tools” tab and navigate to “Detector Manager”
 - a. Click on the “File” dropdown and select “New”.
 - b. Name the target as “N1”
 - i. Set the reporter dye to “ROX”
 - ii. Set the quencher dye to none
 - iii. Click Apply
 - c. Name the target as “RNaseP”
 - i. Set the reporter dye to “FAM”
 - ii. Set the quencher dye to “None”
 - iii. Click Apply
 - d. Name the target as “IAC”
 - i. Set the reporter dye to “Cy5”
 - ii. Set the quencher dye to “None”
 - iii. Click Apply
3. Click the “File” tab and select “New”
4. On the “Define document” Pop-Up:
 - a. Assay: Standard Curve (Absolute Quantification)
 - b. Container: 96 Well Clear
 - c. Template: Blank Document
 - d. Run Mode: Fast 7500
 - e. Operator: Select the operator you wish to run the assay with
 - f. Plate Name: Select your desired name for the experiment file
 - g. Click “Next”
5. Select the previously created detectors from the library: N1, RNaseP, IAC and click add.
6. Set the Passive Reference to “None”
7. Click “next”. Select all the wells and assign the targets to them.
8. Select Finish.
9. Navigate to the Instrument tab.
 - a. Change the Sample Volume to **15.0 µL**
 - b. Click the “Add Hold” button until you have two hold steps. Set the number of repetitions to 1.
 - c. Click the “Add Step” button until you have three PCR cycling steps. Set the number of repetitions for this stage to 40.
 - d. Going back to the first hold stage enter, the following parameters (**Table C1**):
 - i. Hold Stage: Step 1
 1. Auto ramp rate
 2. 46°C
 3. 20 minutes
 - ii. Hold Stage: Step 2

1. Auto ramp rate
 2. 95°C
 3. 5 minutes
 - iii. PCR Stage: Step 1
 1. Auto ramp rate
 2. 95°C
 3. 3 seconds
 - iv. PCR Stage: Step 2
 1. Auto ramp rate
 2. 62°C
 3. 24 seconds.
 - v. PCR Stage: Step 3
 1. Auto ramp rate
 2. 70°C
 3. 5 seconds
 - vi. Under settings, select the following:
 1. Sample Volume: 20
 2. Run Mode: Fast 7500
 3. Data Calculation: Stage 3, step 2
- e. Select the “Analysis” tab and click “Analysis settings”.
 - i. Select N1’s target settings by clicking on the detector dropdown.
 1. Select “Manual CT” and **set the threshold to 50,000**
 2. Select “Manual Baseline” and set the start cycle to 3 and the end to 10.
 3. Click apply
 - ii. Select RNaseP
 1. Select Manual CT and **set the threshold to 100,000**
 2. Select “Manual Baseline” and set the start cycle to 3 and the end to 15
 3. Click apply
 - iii. Select IAC
 1. Select Manual CT and **set the threshold to 100,000**
 2. Select “Manual Baseline” and set the start cycle to 3 and the end to 20
 3. Click “Apply”, then select “OK”.
- f. Select File -> Save. Set your desired name and your template is saved.

APPENDIX D: Bio-Rad CFX96 Touch™ Programing

Direct Patient Sample (20 µL reaction volume)

Please refer to the following steps for **CFX96 Touch™** manual programming specific for Direct Patient sample testing.

1. Launch the CFX Maestro software.
2. Select “File”, then choose “New” and select “Protocol”
3. Program thermocycling conditions in the Protocol Editor
 - a. Set Sample Volume to **20 µL**
 - b. Click “Insert Step” to add a total of five total steps and program as follows (**Table D1**):
 - c. Step 1
 - i. Temp: 46°C
 - ii. Time: 20:00
 - d. Step 2
 - i. Temp: 95°C
 - ii. Time: 5:00
 - e. Step 3
 - i. Temp: 95°C
 - ii. Time: 0:03
 - f. Step 4
 - i. Temp: 62°C
 - ii. Time: 0:20
 - iii. Click “Add Plate Read to Step”. Also, ensure this is the only step containing a plate read
 - g. Step 5
 - i. Temp: 70°C
 - ii. Time: 0:05
 - h. Step 6
 - i. Click to highlight “GOTO” and enter “3” to indicate thermocycling includes Step 3, Step 4 and Step 5.
 - ii. At “GOTO 3”, confirm “39 more times” is programmed for a total of 40 cycles.
 - I. Save the new cycling conditions as protocol for future use
 - i. At the upper left of the screen select the Save button
 - ii. Save in the ExpressLoad folder
 - iii. Name the file ‘500003XMPDirectCFX96v2’
 - iv. Save as type ‘Protocol File (*.prcl)’
 - v. Select Save
 - vi. Click Ok in the protocol editor window

Table D1. CFX96 Touch™ thermocycling summary - Direct Patient Sample.

| PCR Cycle Summary | | | | | |
|--------------------|------------|--------|-----------|-----------|--------|
| | Hold Stage | | PCR Stage | | |
| Conditions | Step 1 | Step 2 | Step 1 | Step 2 | Step 3 |
| Temperature (°C/s) | 46 | 95 | 95 | 62 | 70 |
| Duration (Min:sec) | 20:00 | 5:00 | 0:03 | 0:20 | 0:05 |
| Detection | OFF | OFF | OFF | ON | OFF |
| No. of PCR Cycles | HOLD | HOLD | 40 | | |

4. Define the plate setup

- a. Select “File” then choose “New” and select “Plate”
- b. In the Plate Editor, click settings and ensure your settings are as follows
 - i. Plate Size: 96 wells
 - ii. Plate Type: BR White OR BR Clear (BioGX recommends BR White)
- c. Highlight all wells and choose “Select Fluorophores”
 - i. Uncheck all currently selected wells and check ONLY the following: FAM, Cal Red 610, Quasar 670
- d. For “Sample Type” select “Unknown”
- e. Check the boxes besides all three of your selected fluorophores. Change the target name as follows:
 - i. FAM: RNaseP
 - ii. Cal Red 610: N1
 - iii. Quasar 670: IAC
- f. Save the new plate setup for future use
 - i. At the upper left of the screen select “File” then select “Save”
 - iii. Name the file ‘500003XMPDirect_plateCFX96v2’
 - iv. Save as type ‘Plate File (*.pltd)’
 - v. Select Save
 - vi. Click Ok in the Plate Editor window
- g. Saving Protocol
 - i. Select “File” then “Open”
 - ii. Select “Protocol” then open your saved protocol.
 - ii. Ensure the PCR steps are correct and click “OK”
 - iii. Click the “Plate” tab and choose “Select Existing”. Choose the plate that was saved.
 - iv. Add any desired notes and click on the “Start Run” tab.
 - v. Save the run with the desired name
- h. Assigning a sample name to well positions

Once the run has begun, reaction wells can be assigned with a unique sample name as follows:

- i. Navigate to “Real Time Status”
 - ii. On the top-middle section of the screen, click “Plate Set-Up” followed by “View/Edit Plate”.
 - iii. Click “Spreadsheet View/Importer” on the upper right quadrant of the screen. Wells can be named with unique identifiers for Sample Name, Sample Type, etc.
5. When CFX96 Touch™ run is completed, threshold setting update will need to be completed as follows:
- a. Open the “Data Analysis” window for your specific run
 - b. To apply threshold settings for FAM-channel, uncheck CalRed 610-channel and Quasar 670-channel and be sure “**FAM**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for FAM will apply to the experiment.
 - c. To apply threshold settings for CalRed 610-channel, uncheck FAM-channel and Quasar 670-channel and be sure “**CalRed 610**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for CalRed 610 will apply to the experiment
 - d. To apply threshold settings for Quasar 670-channel, uncheck FAM-channel and CalRed 610-channel and be sure “**Quasar 670**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “**Baseline Threshold**”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for Quasar 670 will apply to the experiment

Bio-Rad CFX96 Touch™ Programing

Extracted Patient Sample (15 µL reaction volume)

Please refer to the following steps for CFX96 Touch™ manual programming specific for Extracted Patient sample testing.

1. Launch the CFX Maestro software.
2. Choose “File”, then choose “New” and select “Protocol”
3. Program thermocycling conditions in the Protocol Editor
 - a. Set Sample Volume to **15 µL**
 - b. Click “Insert Step” to add a total of five total steps and program as follows (**Table D1**):
 - c. Step 1
 - i. Temp: 46°C
 - ii. Time: 20:00
 - d. Step 2
 - i. Temp: 95°C
 - ii. Time: 5:00
 - e. Step 3
 - i. Temp: 95°C
 - ii. Time: 0:03
 - f. Step 4
 - i. Temp: 62°C
 - ii. Time: 0:20
 - iii. Click “Add Plate Read to Step”. Also, ensure this is the only step containing a plate read
 - g. Step 5
 - i. Temp: 70°C
 - ii. Time: 0:05
 - h. Step 6
 - i. Click to highlight “GOTO” and enter “3” to indicate thermocycling includes Step 3, Step 4 and Step 5.
 - ii. At “GOTO 3”, confirm “39 more times” is programmed for a total of 40 cycles.
 - i. Save the new cycling conditions as a protocol for future use
 - i. At the upper left of the screen select the Save button
 - ii. Save in the ExpressLoad folder
 - iii. Name the file ‘500003XMPExtractCFX96v2’
 - iv. Save as type ‘Protocol File (*.prcl)’
 - v. Select Save
 - vi. Click Ok in the protocol editor window
4. Define the plate setup
 - a. Select “File” then choose “New” and select “Plate”
 - b. In the Plate Editor, click settings and ensure your settings are as follows
 - i. Plate Size: 96 wells
 - ii. Plate Type: BR White OR BR Clear (BioGX recommends BR White)

- c. Highlight all wells and choose “Select Fluorophores”
 - i. Uncheck all currently selected wells and check ONLY the following: FAM, Cal Red 610, Quasar 670
 - d. For “Sample Type” select “Unknown”
 - e. Check the boxes besides all three of your selected fluorophores. Change the target name as follows:
 - i. FAM: RNaseP
 - ii. CalRed 610: N1
 - iii.: Quasar 670: IAC
 - f. Save the new plate setup for future use
 - i. At the upper left of the screen select “File” then select “Save”
 - iii. Name the file “500003XMPEXtract_plateCFX96v2”.
 - iv. Ensure it is saved in the “*.pltd” format
 - v. Select Save
 - vi. Click Ok in the Plate Editor window
 - g. Saving Protocol
 - i. Select “File” then “Open”
 - ii. Select “Protocol” then open your saved protocol.
 - ii. Ensure the PCR steps are correct and click “OK”
 - iii. Click the “Plate” tab and choose “Select Existing”. Choose the plate that was saved.
 - iv. Add any desired notes and click on the “Start Run” tab.
 - v. Save the run with the desired name
 - h. Assigning a sample name to well positions

Once the run has begun, reaction wells can be assigned with a unique sample name as follows:

 - i. Navigate to “Real Time Status”
 - ii. On the top-middle section of the screen, click “Plate Set-Up” followed by “View/Edit Plate”.
 - iii. Click “Spreadsheet View/Importer” on the upper right quadrant of the screen. Wells can be named with unique identifiers for Sample Name, Sample Type, etc.
5. When CFX96 Touch™ run is completed, threshold setting update will need to be completed as follows:
- a. Open the “Data Analysis” window for your specific run
 - b. To apply threshold settings for FAM-channel, uncheck CalRed 610-channel and Quasar 670-channel and be sure “**FAM**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for FAM will apply to the experiment.
 - c. To apply threshold settings for CalRed 610-channel, uncheck FAM-channel and Quasar 670-channel and be sure “**CalRed 610**” is checked (Note: Threshold

- adjustments can be performed only in single threshold mode, be sure only one dye is selected)
- i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for CalRed 610 will apply to the experiment
- d. To apply threshold settings for Quasar 670-channel, uncheck FAM-channel and CalRed 610-channel and be sure “**Quasar 670**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
- i. Navigate to “Settings” and select “**Baseline Threshold**”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for Quasar 670 will apply to the experiment

APPENDIX E: Bio-Rad CFX384 Touch™ Programming

Direct Patient Sample (20 µL reaction volume)

Please refer to the following steps for **CFX384 Touch™** manual programming specific for Direct Patient sample testing.

1. Launch the CFX Maestro software.
2. Select “File”, then choose “New” and select “Protocol”
3. Program thermocycling conditions in the Protocol Editor
 - a. Set Sample Volume to **20 µL**
 - b. Click “Insert Step” to add a total of five total steps and program as follows (**Table E1**):
 - c. Step 1
 - i. Temp: 46°C
 - ii. Time: 20:00
 - d. Step 2
 - i. Temp: 95°C
 - ii. Time: 5:00
 - e. Step 3
 - i. Temp: 95°C
 - ii. Time: 0:03
 - f. Step 4
 - i. Temp: 62°C
 - ii. Time: 0:20
 - iii. Click “Add Plate Read to Step”. Also, ensure this is the only step containing a plate read
 - g. Step 5
 - i. Temp: 70°C
 - ii. Time: 0:05
 - h. Step 6
 - i. Click to highlight “GOTO” and enter “3” to indicate thermocycling includes Step 3, Step 4 and Step 5.
 - ii. At “GOTO 3”, confirm “39 more times” is programmed for a total of 40 cycles.
 - I. Save the new cycling conditions as protocol for future use
 - i. At the upper left of the screen select the Save button
 - ii. Save in the ExpressLoad folder
 - iii. Name the file ‘500003XMPDirectCFX384’
 - iv. Save as type ‘Protocol File (*.prcl)’
 - v. Select Save
 - vi. Click Ok in the protocol editor window

Table E1. CFX384 Touch™ thermocycling summary - Direct Patient Sample.

| PCR Cycle Summary | | | | | |
|--------------------|------------|--------|-----------|-----------|--------|
| | Hold Stage | | PCR Stage | | |
| Conditions | Step 1 | Step 2 | Step 1 | Step 2 | Step 3 |
| Temperature (°C/s) | 46 | 95 | 95 | 62 | 70 |
| Duration (Min:sec) | 20:00 | 5:00 | 0:03 | 0:20 | 0:05 |
| Detection | OFF | OFF | OFF | ON | OFF |
| No. of PCR Cycles | HOLD | HOLD | 40 | | |

4. Define the plate setup

- a. Select “File” then choose “New” and select “Plate”
- b. In the Plate Editor, click settings and ensure your settings are as follows
 - i. Plate Size: 384 well
 - ii. Plate Type: BR White OR BR Clear (BioGX recommends BR White)
- c. Highlight all wells and choose “Select Fluorophores”
 - i. Uncheck all currently selected wells and check ONLY the following: FAM, Cal Red 610, Quasar 670
- d. For “Sample Type” select “Unknown”
- e. Check the boxes besides all three of your selected fluorophores. Change the target name as follows:
 - i. FAM: RNaseP
 - ii. Cal Red 610: N1
 - iii. Quasar 670: IAC
- f. Save the new plate setup for future use
 - i. At the upper left of the screen select “File” then select “Save”
 - iii. Name the file ‘500003XMPDirect_plateCFX384’
 - iv. Save as type ‘Plate File (*.pltd)’
 - v. Select Save
 - vi. Click Ok in the Plate Editor window
- g. Saving Protocol
 - i. Select “File” then “Open”
 - ii. Select “Protocol” then open your saved protocol.
 - ii. Ensure the PCR steps are correct and click “OK”
 - iii. Click the “Plate” tab and choose “Select Existing”. Choose the plate that was saved.
 - iv. Add any desired notes and click on the “Start Run” tab.
 - v. Save the run with the desired name
- h. Assigning a sample name to well positions

Once the run has begun, reaction wells can be assigned with a unique sample name as follows:

- i. Navigate to “Real Time Status”
 - ii. On the top-middle section of the screen, click “Plate Set-Up” followed by “View/Edit Plate”.
 - iii. Click “Spreadsheet View/Importer” on the upper right quadrant of the screen. Wells can be named with unique identifiers for Sample Name, Sample Type, etc.
5. When CFX96 Touch™ run is completed, threshold setting update will need to be completed as follows:
- a. Open the “Data Analysis” window for your specific run
 - b. To apply threshold settings for FAM-channel, uncheck CalRed 610-channel and Quasar 670-channel and be sure “**FAM**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for FAM will apply to the experiment.
 - d. To apply threshold settings for CalRed 610-channel, uncheck FAM-channel and Quasar 670-channel and be sure “**CalRed 610**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for CalRed 610 will apply to the experiment
 - e. To apply threshold settings for Quasar 670-channel, uncheck FAM-channel and CalRed 610-channel and be sure “**Quasar 670**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “**Baseline Threshold**”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for Quasar 670 will apply to the experiment

Bio-Rad CFX384 Touch™ Programming

Extracted Patient Sample (15 µL reaction volume)

Please refer to the following steps for **CFX384 Touch™** manual programming specific for Extracted Patient sample testing.

1. Launch the CFX Maestro software.
2. Choose “File”, then choose “New” and select “Protocol”
3. Program thermocycling conditions in the Protocol Editor
 - a. Set Sample Volume to **15 µL**
 - b. Click “Insert Step” to add a total of five total steps and program as follows (**Table E1**):
 - c. Step 1
 - i. Temp: 46°C
 - ii. Time: 20:00
 - d. Step 2
 - i. Temp: 95°C
 - ii. Time: 5:00
 - e. Step 3
 - i. Temp: 95°C
 - ii. Time: 0:03
 - f. Step 4
 - i. Temp: 62°C
 - ii. Time: 0:20
 - iii. Click “Add Plate Read to Step”. Also, ensure this is the only step containing a plate read
 - g. Step 5
 - i. Temp: 70°C
 - ii. Time: 0:05
 - h. Step 6
 - i. Click to highlight “GOTO” and enter “3” to indicate thermocycling includes Step 3, Step 4 and Step 5.
 - ii. At “GOTO 3”, confirm “39 more times” is programmed for a total of 40 cycles.
 - i. Save the new cycling conditions as a protocol for future use
 - i. At the upper left of the screen select the Save button
 - ii. Save in the ExpressLoad folder
 - iii. Name the file ‘500003XMPExtractCFX384’
 - iv. Save as type ‘Protocol File (*.prcl)’
 - v. Select Save
 - vi. Click Ok in the protocol editor window
4. Define the plate setup
 - a. Select “File” then choose “New” and select “Plate”
 - b. In the Plate Editor, click settings and ensure your settings are as follows
 - i. Plate Size: 384 well
 - ii. Plate Type: BR White OR BR Clear (BioGX recommends BR White)

- c. Highlight all wells and choose “Select Fluorophores”
 - i. Uncheck all currently selected wells and check ONLY the following: FAM, Cal Red 610, Quasar 670
 - d. For “Sample Type” select “Unknown”
 - e. Check the boxes besides all three of your selected fluorophores. Change the target name as follows:
 - i. FAM: RNaseP
 - ii. CalRed 610: N1
 - iii.: Quasar 670: IAC
 - f. Save the new plate setup for future use
 - i. At the upper left of the screen select “File” then select “Save”
 - iii. Name the file “500003XMPExtract_plateCFX384”.
 - iv. Ensure it is saved in the “*.pltd” format
 - v. Select Save
 - vi. Click Ok in the Plate Editor window
 - g. Saving Protocol
 - i. Select “File” then “Open”
 - ii. Select “Protocol” then open your saved protocol.
 - ii. Ensure the PCR steps are correct and click “OK”
 - iii. Click the “Plate” tab and choose “Select Existing”. Choose the plate that was saved.
 - iv. Add any desired notes and click on the “Start Run” tab.
 - v. Save the run with the desired name
 - h. Assigning a sample name to well positions

Once the run has begun, reaction wells can be assigned with a unique sample name as follows:

 - i. Navigate to “Real Time Status”
 - ii. On the top-middle section of the screen, click “Plate Set-Up” followed by “View/Edit Plate”.
 - iii. Click “Spreadsheet View/Importer” on the upper right quadrant of the screen. Wells can be named with unique identifiers for Sample Name, Sample Type, etc.
5. When CFX96 Touch™ run is completed, threshold setting update will need to be completed as follows:
- a. Open the “Data Analysis” window for your specific run
 - b. To apply threshold settings for FAM-channel, uncheck CalRed 610-channel and Quasar 670-channel and be sure “**FAM**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
 - i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for FAM will apply to the experiment.
 - d. To apply threshold settings for CalRed 610-channel, uncheck FAM-channel and Quasar 670-channel and be sure “**CalRed 610**” is checked (Note: Threshold

- adjustments can be performed only in single threshold mode, be sure only one dye is selected)
- i. Navigate to “Settings” and select “Baseline Threshold”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for CalRed 610 will apply to the experiment
- e. To apply threshold settings for Quasar 670-channel, uncheck FAM-channel and CalRed 610-channel and be sure “**Quasar 670**” is checked (Note: Threshold adjustments can be performed only in single threshold mode, be sure only one dye is selected)
- i. Navigate to “Settings” and select “**Baseline Threshold**”
 - ii. At the bottom of the popup window under “single threshold”, select “User Defined” and enter “**500**”
 - iii. Click “OK” and the threshold for Quasar 670 will apply to the experiment

APPENDIX F: BioGX pixl.16 Programing

Direct Patient Sample (20 µL reaction volume)

Please refer to the following steps for pixl.16 touchpad manual programming specific for Direct patient sample testing.

1. Open the “BioGX qPCR” pixl app(Operating software: version 1.6.9)
2. Select “Test” on the left side home screen
3. Within the “Test” screen, tap “New Experiment” to proceed to the Sample Setup screen
4. In the Experiment Name section, enter the test name.
 - a. Experiment Name: User-defined or 500003XMPDirectpixlRev01
5. In the Channel section, select the dye and define the target identified in each channel.
 - a. Select Channel 1
 - i. From the drop-down menu select: FAM
 - ii. Name the Channel: RNaseP
 - b. Select Channel 3
 - i. From the drop-down menu select: ROX
 - ii. Name the Channel: CoV2-N1
 - c. Select Channel 4
 - i. From the drop-down menu select: Cy5
 - ii. Name the Channel: IAC
 - d. Integration Time for pixl optimized tests are recommended to remain set to: auto.
 - e. To name the wells by sample name, select the appropriate well and next to “Name” rename well to whatever is desired for the experiment.
 - f. If necessary, a barcode scanner can be plugged into the USB port located on the front panel of the instrument to facilitate sample ID capture.
6. Tap Next to go to the Cycling Program screen
7. Add, remove, or edit a stage or step.
8. Adjust the temperature and time of a stage of step, number of cycles, and data capture step.
 - a. Ensure Auto Integration Time is selected
 - b. Within the section of the thermocycling parameters, an additional step will need to be added by selecting the “+” next to “Const-tempe Stage”.
 - c. Starting at the first “Pre-Denat” stage enter the following parameters (**Table F1**)
 - i. Pre-Denat: Step 1
 1. 46°C

2. 600 seconds
 - ii. Pre-Denat2: Step 1
 1. 95°C
 2. 150 seconds
 - iii. Cycle Period: Step 1
 1. 95°C
 2. 5 seconds
 - iv. Cycle Period: Step 2
 1. 59°C
 2. 12 seconds
 - v. Hold: Step 1
 1. 50°C
 2. 60 seconds
- d. Save the template file before starting the run by clicking “Save Template” in the upper right corner.

Table F1: BioGX pixl.16 thermocycling summary: Direct Patient Samples.

| PCR Cycle Summary | | | | | |
|-------------------|-----------------|--------|--------------------|--------|------------|
| | Pre-Denat Stage | | Cycle Period Stage | | Hold Stage |
| Conditions | Step 1 | Step 2 | Step 1 | Step 2 | Step 1 |
| Temperature (°C) | 46 | 95 | 95 | 59 | 50 |
| Duration (sec) | 600 | 150 | 5 | 12 | 60 |
| Image Step | No | No | No | Yes | No |
| No. of PCR Cycles | 1 | 1 | 40 | | 1 |

9. Start Run

- a. Select Start in the bottom right corner of the touchpad, this will automatically take you to the “Run” tab

10. After run completion, navigate to the “Results” tab and select the appropriate test to set the threshold for each channel used

- a. Set Ct Low Limit to 13
- b. Set Ct Threshold (%) to 6.0

11. Save File (Report tab)

- a. In the upper right corner, select “Export” to save .json file, name file as desired or “500003XMPDirectpixlRev01”
- b. Insert a USB key in the USB port located on the front panel of the instrument to save the results file
- c. Select “CSV” to save the file as an excel document
- d. Select “PDF” to save the file as a PDF document
- e. Select “JSON” to save the file as a JSON document

BioGX pixl.16 Programming

Extracted Patient Sample (15 µL reaction volume)

Please refer to the following steps for pixl.16 touchpad manual programming specific for Extracted patient sample testing.

1. Open the “BioGX qPCR pixl” app(Operating software: version 1.6.9)
2. Select “Test” on the left side home screen
3. Within the “Test” screen, tap “New Experiment” to proceed to the Sample Setup screen
4. In the Experiment Name section, enter the test name.
 - a. Experiment Name: User-defined or 500003XMPDirectpixlRev01
5. In the Channel section, select the dye and define the target identified in each channel.
 - a. Select Channel 1
 - i. From the drop-down menu select: FAM
 - ii. Name the Channel: RNaseP
 - b. Select Channel 3
 - i. From the drop-down menu select: ROX
 - ii. Name the Channel: CoV2-N1
 - c. Select Channel 4
 - i. From the drop-down menu select: Cy5
 - ii. Name the Channel: IAC
 - d. Integration Time for pixl optimized tests are recommended to remain set to: auto.
 - e. To name the wells by sample name, select the appropriate well and next to “Name” rename well to whatever is desired for the experiment.
 - f. If necessary, a barcode scanner can be plugged into the USB port located on the front panel of the instrument to facilitate sample ID capture.
6. Tap Next to go to the Cycling Program screen
7. Add, remove, or edit a stage or step.
8. Adjust the temperature and time of a stage of step, number of cycles, and data capture step.
 - a. Ensure Auto Integration Time is selected
 - b. Within the section of the thermocycling parameters, an additional step will need to be added by selecting the “+” next to “Const-tempe Stage”.
 - c. Starting at the first “Pre-Denat” stage enter the following parameters (**Table F1**)
 - i. Pre-Denat: Step 1
 1. 46°C

2. 600 seconds
 - ii. Pre-Denat2: Step 1
 1. 95°C
 2. 150 seconds
 - iii. Cycle Period: Step 1
 1. 95°C
 2. 5 seconds
 - iv. Cycle Period: Step 2
 1. 59°C
 2. 12 seconds
 - v. Hold: Step 1
 1. 50°C
 2. 60 seconds
 - d. Save the template file before starting the run by clicking “Save Template” in the upper right corner.
9. Start Run
- a. Select Start in the bottom right corner of the touchpad, this will automatically take you to the “Run” tab
10. After run completion, navigate to the “Results” tab and select the appropriate test to set the threshold for each channel used
- a. Set Ct Low Limit to 13
 - b. Set Ct Threshold (%) to 6.0
11. Save Results File (Report tab)
- a. In the upper right corner, select “Export” to save .json file, name file as desired or “500003XMPExtractedpixlRev01”
 - b. Insert a USB key in the USB port located on the front panel of the instrument to save the results file
 - c. Select “CSV” to save the file as an excel document
 - d. Select “PDF” to save the file as a PDF document
 - e. Select “JSON” to save the file as a JSON document

APPENDIX G: Bio Molecular Systems Mic Instrument Programing

Direct Patient Sample (20 µL reaction volume)

Please refer to the following steps for **Bio Molecular Systems Mic Instrument** manual programming specific for Direct Patient sample testing.

1. Launch the Mic PCR software. (Operating software: version 2.8)
2. In the top left of the software, select “New”
 - a. Select “Assay”
3. Select the “Information” tab on the banner on the left side of the software.
4. Under “Chemistry type” select “Hydrolysis Probes”
5. In the “Targets” section:
 - a. Enter “RNase P” as the first target and select FAM as the Reporter Dye
 - b. Enter “N1” as the second target and select “Cal Fluor 610” as the Reporter Dye.
 - c. Enter “IAC” as the third target and select “Q670” as the Reporter Dye.
6. The remaining fields in the “Information” tab do not need to be populated.
7. Select “Profile”
 - a. On the top right of the software banner, select “Fast Taq” for the Temperature Control.
 - b. Set the volume to **20 µL** for direct sample addition testing.
8. Thermocycling programming parameters (**Table G1**)
 - a. Pre-Denat: Step 1
 - i. 46°C
 - ii. 1200 seconds
 - b. Pre-Denat2: Step 1
 - i. 95°C
 - ii. 300 seconds
 - c. Cycle Period: Step 1
 - i. 95°C
 - ii. 3 seconds
 - d. Cycle Period: Step 2
 - i. 62°C
 - ii. 20 seconds
 - iii. Select the camera to turn on acquisition.
 - e. Cycle Period: Step 3

- i. 70°C
 - ii. 5 seconds
- f. Set the number of cycles to 40.
9. In the top left of the software select “Save As”
 - a. Name the file as desired. The file extension should be “.micassay”
10. Start run

Table G1. Bio Molecular Systems Mic Instrument thermocycling summary: Direct Patient Samples.

| PCR Cycle Summary | | | | | |
|-------------------|-----------------|--------|--------------------|--------|--------|
| | Pre-Denat Stage | | Cycle Period Stage | | |
| Conditions | Step 1 | Step 2 | Step 1 | Step 2 | Step 3 |
| Temperature (°C) | 46 | 95 | 95 | 62 | 70 |
| Duration (sec) | 1200 | 300 | 3 | 20 | 5 |
| Image Step | No | No | No | Yes | No |
| No. of PCR Cycles | 1 | 1 | 40 | | |

Bio Molecular Systems Mic Instrument - Thermocycling Programming

Extracted Patient Sample (15 µL reaction volume)

Please refer to the following steps for **Bio Molecular Systems Mic Instrument** manual programming specific for Direct Patient sample testing.

1. Launch the Mic PCR software (Operating software: version 2.8).
2. In the top left of the software, select “New”
 - a. Select “Assay”
3. Select the “Information” tab on the banner on the left side of the software.
4. Under “Chemistry type” select “Hydrolysis Probes”
5. In the “Targets” section:
 - a. Enter “RNase P” as the first target and select FAM as the Reporter Dye
 - b. Enter “N1” as the second target and select “Cal Fluor 610” as the Reporter Dye.
 - c. Enter “IAC” as the third target and select “Q670” as the Reporter Dye.
6. The remaining fields in the “Information” tab do not need to be populated.
7. Select “Profile” tab located on the software banner.
 - a. On the top right of the software banner, select “Fast Taq” for the Temperature Control.
 - b. Set the volume to **15 µL** for direct sample addition testing.
8. Thermocycling programming parameters (**Table G1**)
 - a. Pre-Denat: Step 1
 - i. 46°C
 - ii. 1200 seconds
 - b. Pre-Denat2: Step 1
 - i. 95°C
 - ii. 300 seconds
 - c. Cycle Period: Step 1
 - i. 95°C
 - ii. 3 seconds
 - d. Cycle Period: Step 2
 - i. 62°C
 - ii. 20 seconds
 - iii. Select the camera to turn on acquisition.
 - e. Cycle Period: Step 3

- i. 70°C
 - ii. 5 seconds
 - f. Set the number of cycles to 40.
9. In the top left of the software select “Save As”
 - a. Name the file as desired. The file extension should be “.micassay”
10. Start run

APPENDIX H: EUO LABELING

For the Applied Biosystems QuantStudio 5 (0.2 mL 96-well and 384-well), Bio-Rad CFX96 Touch, Bio-Rad CFX384 Touch PCR, Bio Molecular Systems Mic and BioGX pixl.16 Instruments

Please print the below label and place the label on the front panel of the instrument. If the instrument includes labeling indicating “For Research Use Only”, please cover with the below “Emergency Use Only” labeling. The instrument should retain this labeling throughout the EUA use of the BioGX Xfree™ COVID-19 Direct RT-PCR assay.

 **Emergency Use Only**

This instrument is authorized for use with the
BioGX Xfree™ COVID-19 Direct RT-PCR assay.














REVISION HISTORY

| Version | Date | Description of Change |
|------------|-------------|---|
| 04.5 | 1 NOV 2022 | Update of expiration date to 15 months from date of manufacture, Update with the addition of BioGX pixl Real-Time PCR platform for detection of direct addition of individual samples and detection of nucleic acid extracted individual samples, Update with the addition of Bio Molecular Systems Mic Instrument for detection of direct addition of individual samples and detection of nucleic acid extracted individual samples. Update with the addition of Applied Biosystems QuantStudio 5 (384-well) platform for detection of direct addition of individual samples and detection of nucleic acid extracted individual samples, Update with the addition of BioGX pixl Real-Time PCR platform and consumables part numbers, Update with the addition of Bio Molecular Systems Mic Instrument consumable part numbers, Update with the addition of Applied Biosystems QuantStudio 5 (384-well) platform consumable part numbers, |
| 04.2 | 30 JUN 2021 | Remove “Draft” from Revision 04.1 |
| Draft 04.1 | 22 JUN 2021 | Addition of Appendix E –EUO Label for platforms Accepted FDA text edit suggestions |
| Draft 04 | 14 JUN 2021 | FDA redline text suggestions accepted. Added Table of Contents section. Updated Materials Required but not provided section to include pipettes, pipette tips, centrifuge, and 1.5 mL tubes. Added FDA recommended additional warnings and precautions. Updated external positive control run frequency to one external positive control be included with each RT-PCR run of patient samples and external negative controls should be run with each extraction batch (as applicable) and each PCR run. Accepted FDA recommended clinical performance. Specimen collection, storage, and handling section added. Added information for customers on how to obtain EUO label for platforms |

| | | |
|------------|-------------|--|
| | | Minor text edits and formatting |
| Draft 03.1 | 26 MAR 2021 | <p>Update number of reactions (tests) per tube to number of reactions (tests) per kit. Update Table 2 to simplify interpretation, Revision status updated to 03.1 from 03</p> <p>FDA Recommended Track Changes (28MAY2021)-Update “Preparation of BioGX External Controls” section, update Table. 1 to simplify expected Ct values for N1 and IAC.</p> <p>FDA Recommended Track Changes (08JUN2021)- Update of storage of rehydrated master mix to indicate unused rehydrated master mix is to be discarded</p> <p>Update “Preparation of BioGX External Controls” section to include “Preparation of BioGX External Controls for Direct Sample Workflow”</p> <p>Update “Quality Control” section to include Zepetometrix SARS-CoV-2 as alternative positive control</p> <p>Update dilution protocol in “Preparation of BioGX External Controls” section</p> |
| 03 | 10 FEB 2021 | Update fluorophore utilized for IAC detection, addition of nasopharyngeal wash/aspirates and nasal aspirate sample types, addition of Applied BioSystems 7500 Fast Dx and Bio-Rad CFX384 Touch platforms, update BioGX Rehydration Water Part number to 800-0035-12, update use of Qiagen Viral RNA Mini Kit, update comparator method (Linea™ COVID-19 assay), update clinical concordance study. Revision status updated to 02.4 from 03 |
| 02.4 | 19 NOV 2020 | Correction of revision date, “ <i>Validation of this test has not been reviewed by FDA</i> ” paragraph moved to the end of the Intended Use statement on Page 2. Revision status updated to 02.4 from 02.3 |
| 02.3 | 19 NOV 2020 | Intended Use statement on Page 2 updated to include “ <i>Validation of this test has not been reviewed by FDA</i> ” paragraph. Revision status updated to 02.3 from 02.2 |
| 02.2 | 18 NOV 2020 | Update FDA EUA Review Status statement, Update “Limitations of the Procedure” section, update “Clinical Performance” section, update “Reference” section. Revision status updated to 02.2 from 02.1 |
| 02.1 | 12 NOV 2020 | Addition of FDA EUA Review Status, update positive control and patient result interpretation, update limit of detection table, update clinical performance. Revision status updated to 02.1 from 02 |

| | | |
|----|-------------|--|
| 02 | 20 OCT 2020 | Correction of BioGX address, minor formatting changes, correction of references, correction of positive control use. Revision status updated to 02 from 01 |
| 01 | 09 OCT 2020 | Initial Release |

SYMBOLS

| Symbol | Meaning |
|---|---|
|  | Catalog number |
|  | <i>In vitro</i> diagnostic medical device |
|  | Do not reuse |
|  | Batch code |
|  | Caution |
|  | Consult instructions for use |
|  | Manufacturer |
|  | Contains sufficient for <n> tests |
|  | Temperature limitation |
|  | Keep dry |
|  | Keep away from sunlight |
|  | Expiration date |
|  | Biological Risks |

| | |
|----------------|---------|
| CONTROL | Control |
|----------------|---------|

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Xfree COVID-19 Direct RT-PCR

REF 500-003-XMP

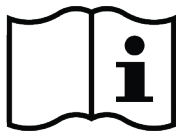
R_x Only IVD

For use under the Emergency Use (EUA) Only

This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3 (b)(1), unless the declaration is terminated or authorization is revoked sooner.



Be advised, this is not the complete product IFU. Consult current version of Instructions For Use (IFU) and download current version of Electronic User Defined Protocol (eUDP) prior to use. Current versions are available at www.biogx.com OR by emailing info@biogx.com for an electronic version OR a free printed copy.



Current IFU version: **500-003-XMP Xfree™ COVID-19 Direct RT-PCR (US) Rev.Draft03.1**
Current eUDP version:

ABI 7500 Fast Dx:
Direct: 500003XMPDirect7500Dx / 500003XMPDirect7500Dx.sdt
Extracted: 500003XMPExtract7500Dx / 500003XMPExtract7500Dx.sdt

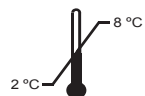
Bio-Rad CFX96 Touch™:
Direct: 500003XMPDirect_plateCFX96v2.pltd / 500003XMPDirectCFX96v2 / 500003XMPDirectCFX96v2.prcl
Extracted: 500003XMPExtract_plateCFX96v2.pltd / 500003XMPExtractCFX96v2 / 500003XMPExtractCFX96v2.prcl

Bio-Rad CFX384 Touch™:
Direct: 500003XMPDirect_plateCFX384.pltd / 500003XMPDirectCFX384 / 500003XMPDirectCFX384.prcl
Extracted: 500003XMPExtract_plateCFX384.pltd / 500003XMPExtractCFX384 / 500003XMPExtractCFX384.prcl

QuantStudio™ 5:
Direct: 500003XMPDirectQS5v2.edt / 500003XMPDirectQS5v2
Extracted: 500003XMPExtractQS5v2.edt / 500003XMPExtractQS5v2

<https://biogx.com/xfree/>
Download a free QR Code Scanner App with your mobile phone. Using the QR Code App, take a picture of the QR Code. The App will automatically scan the QR code to obtain current versions of IFU and eUDP via the BioGX website.

STORAGE AND STABILITY



BioGX recommends long-term storage at 2-8°C. Product is stable when shipped for under 5 days at ambient temperature.



Reagents have been tested to demonstrate optimal performance when stored properly at 2-8°C and consumed by the expiration date on the kit label.



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.



If pouch is opened by the user but lyophilized reagent is not rehydrated, avoid exposure of lyophilized reagent to moisture and use the entire contents of the opened pouch within 1 month.

SAFE HANDLING

Use clean gloves when handling PCR reagents and buffer tubes.

Do not use reagent tubes if tube caps have been opened or damaged. Do not mix reagents from different pouches and/or kits and/or lots.

