BioGX
Molecular Made Easy

Xfree DNA Master Mix
Open System PCR Reagents

REF **450-051-XMM**

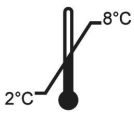
Σ 16 Reactions (Direct Sample)

Σ 64 Reactions (Purified Nucleic Acid)

Product Insert

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

For use with open system Real-Time PCR platforms




RUO

BioGX
1500 First Avenue, North, L136, Birmingham, AL 35203, USA
Phone: +1.205.250.8055
Fax: +1.205.449.8055

For Research Use Only

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

This product is manufactured and packaged as an open system reagent (OSR) for use with open system platforms and has to be validated by the user.

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

PRODUCT OVERVIEW

The BioGX Xfree DNA Master Mix is a pre-mixed, pre-dispensed blend of reagents for performing PCR amplifications. The BioGX Xfree DNA Master Mix is provided as a lyophilized cake and contains reagents necessary to perform PCR amplification, including DNA Polymerase, dNTPs, optimized PCR buffer, and salts. BioGX Xfree DNA Master Mix lyophilized cakes are compatible with direct and purified nucleic acid sample addition.

Reagents in each tube are sufficient to perform 1 x 50 µl PCR reaction with direct sample addition or 4 x 15 µl PCR reactions with purified nucleic acid sample addition.

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

BioGX REF: 450-051-XMM

- Each kit consists of one pouch containing 2 x 8-tube PCR strips.
- Each tube contains one lyophilized BioGX Xfree DNA Master Mix cake sufficient for 1 x 50 µl PCR reaction for direct sample addition workflow or 4 x 15 µl PCR reactions for purified nucleic acid addition workflow.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

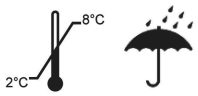
- BioGX Molecular Grade Water or equivalent
    - BioGX Rehydration Water (Part number: 800-0035-12)
- Custom primer and probe or intercalating dye of choice
- Validated Nucleic Acid Extraction Kit (if extracted samples are used as template)
- PCR tubes and/or PCR plates compatible with the PCR instrument of choice
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent
- Microcentrifuge
- Micropipettes and pipette tips
- Disposable nitrile gloves

WARNINGS AND PRECAUTIONS

- For research use only. Not intended for human or animal diagnostics use.
- If handling biological samples, treat as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29[1] and in Biosafety in Microbiological and Biomedical Laboratories[2].
- Do not use the reagents if the protective pouches are open or torn upon arrival.
- Close reagent protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing and store at 2-8 °C.
- Do not remove desiccant from the PCR master mix pouches.
- Do not use master mix if the desiccant is not present or is broken inside the pouches. Do not use reagent vials if they are opened or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not use expired reagents and/or materials.
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where samples or kits are being handled.

- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations.
- Clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water.
- Use clean gloves when handling PCR reagents.

STORAGE REQUIREMENTS AND RECOMMENDATIONS



Reagents are stable at a temperature range of 2-30°C during shipment for 5 days, but BioGX recommends long-term storage at 2-8°C. Reagents have been tested to demonstrate optimal performance when stored properly and consumed by the Manufacturer Recommended Use By Date. The end user may opt to extend the useful life for Research Use Only reagents upon completing performance validations. BioGX's guarantee of reagent integrity does not extend beyond the Manufacturer Recommended Use By Date. Avoid exposing the reagents (lyophilized or rehydrated) to direct sunlight or long-term ambient lighting. Store unused rehydrated master mix up to 24 hours at 2-8°C, protected from light. Tightly reseal the pouch with unused vials and immediately return to a refrigerator after opening. To mitigate reagent performance degradation from exposure to moisture, BioGX suggests using the entire contents of the opened and refrigerated pouch within 1 month; however, the user may choose to verify an extended working time (> 1 month) by performance testing with positive controls and an examination of the sample preparation control target.

QUALITY CONTROL TESTING

Quantified synthetic DNA (BioGX RNase P (DNA template control); Part number: 720-0009) at a concentration of 50 copies/reaction is used for quality control testing.

REAGENT PREPARATION

Please refer to the user manual of the PCR instrument of choice for the generation, import and installation of PCR run files.

1. **Prepare reagents**

a. Keep reagents on ice during set-up.

b. Remove sufficient tubes (1 tube sufficient for single direct sample addition or 1 tube sufficient for 4 samples of purified nucleic acid) of the lyophilized BioGX Xfree DNA Master Mix from their mylar pouch. Return any unused tubes to the mylar pouch, re-seal pouch, and return to the appropriate storage location.

c. Prepare primers/probe or intercalating dye as per manufacturer's instructions. Protect fluorescent probes/intercalating dyes from sunlight or long term exposure to ambient lighting.

d. Prepare template (direct or purified nucleic acid sample) as per manufacturer's instructions.

e. Briefly vortex to thoroughly mix liquid reagents.

f. Quickly centrifuge to collect solutions at the bottom of tubes.

2.1. Procedure for Direct Sample Addition (using liquid primers and probes)

a. Prepare sufficient volume of master mix for the number of PCR reactions needed. **One tube of lyophilized BioGX Xfree DNA Master Mix is sufficient for 1 x 50 µl PCR reaction in direct sample workflow.**

If more than 1 PCR reaction is required, prepare the appropriate number of BioGX Xfree DNA Master Mix tubes, e.g. if 8 PCR reactions are required, prepare 8 BioGX Xfree DNA Master Mix tubes separately (Table 1).

Store unused rehydrated master mix up to 24 hours at 2-8°C, protected from light.

Note: Include controls (no template control and/or positive control) and at least 1 additional reaction to account for pipetting errors.

b. Combine and thoroughly mix all components (**Table 1**). Do not add the sample template. It will be added separately in step 3.

Note: Add sufficient molecular grade water for a final reaction volume of 50 µl with direct sample workflow.

Table 1: PCR Set-Up for use with Direct Sample* addition.

Component	Final concentration or amount	Volume per 50 µl reaction
BioGX Xfree DNA Master Mix	1 cake	N/A
Forward and reverse primers	x nM each	**x µL**
Probe[s] or intercalating dye	y nM each	**y µL**
Direct Sample*	N/A	**20 µL**
Molecular grade water	N/A	**50 µl - x µL - y µL- 20 µL**

*Examples of transport media that support direct sample addition include swab samples transported in Copan UTM, Copan Eswab, 0.85% saline and molecular grade water.

c. Dispense prepared reaction mix **(30 µL for direct sample addition)** into PCR instrument compatible PCR tubes or plates.

d. Continue with step 3.

2.2. Procedure for Purified Nucleic Acid Sample Addition (using liquid primers and probes)

a. Prepare sufficient volume of master mix for the number of PCR reactions needed. **One lyophilized BioGX Xfree DNA Master Mix cake is sufficient for 4 x 15 µL PCR reactions.**

Note: Include controls (no template control and/or positive control) and at least 1 additional reaction to account for pipetting errors.

b. Combine and thoroughly mix all components (**Table 2**). Do not add the sample template. It will be added separately in step 3.

Note: Add sufficient molecular grade water for a final reaction volume of 15 µL after addition of sample template.

Table 2: PCR Set-Up for use with Purified Nucleic Sample Addition.

Component	Final concentration or amount	Volume per 15 µl reaction
BioGX Xfree DNA Master Mix	1 cake	N/A
Forward and reverse primers	x nM each	**x µL**
Probe[s] or intercalating dye	y nM each	**y µL**
Purified Nucleic Acid Sample	N/A	**5 µL**
Molecular grade water	N/A	**15 µl - x µL - y µL - 5 µL**

c. Dispense prepared reaction mix **(10 µL for purified nucleic acid sample addition)** into PCR instrument compatible PCR tubes or plates.

d. Continue with step 3.

2.3. Procedure for direct sample using BioGX lyophilized primers/probe beads

a. Prepare sufficient volume of master mix for the number of PCR reactions needed. **One tube of lyophilized BioGX Xfree DNA Master Mix is sufficient for 1 x 50 µl PCR reaction in direct sample workflow.**

Note: Include controls (no template control and/or positive control) and at least 1 additional reaction to account for pipetting errors.

b. Rehydrate the BioGX forward and reverse primer/probes beads with molecular grade water using the volumes indicated in **Table 3**. Combine and thoroughly mix all components. Do not add the sample template. It will be added separately in step 3.

Note: Add sufficient molecular grade water for a final reaction volume of 50 µL after addition of sample template.

Table 3: PCR Set-Up for use with direct sample addition using BioGX primers/probes

Component	Final concentration or amount	Volume per 50 µl reaction
BioGX Xfree DNA Master Mix	1 cake	N/A
BioGX forward and reverse primers/probes	1 bead X µL rehydration volume	**x µL**
Direct Sample	N/A	**20µL**
Molecular grade water	N/A	**50 µl - x µL - 20 µL**

c. Dispense prepared reaction mix **(30 µL for direct sample addition)** into PCR instrument compatible PCR tubes or plates.

d. Continue with step 3.

2.3. Procedure for Purified Nucleic Acid Sample Addition using BioGX lyophilized primers/probe beads

e. Prepare sufficient volume of master mix for the number of PCR reactions needed. **One tube of lyophilized BioGX Xfree DNA Master Mix is sufficient for 4 x 15 µl PCR reaction in purified nucleic acid sample workflow.**

Note: Include controls (no template control and/or positive control) and at least 1 additional reaction to account for pipetting errors.

f. Rehydrate the BioGX forward and reverse primer/probes beads with molecular grade water using the volumes indicated in **Table 4**. Combine and thoroughly mix all components. Do not add the sample template. It will be added separately in step 3.

Note: Add sufficient molecular grade water for a final reaction volume of 15 µL after addition of sample template.

Table 4: PCR Set-Up for use with purified nucleic acid sample addition using BioGX primers/probes

Component	Final concentration or amount	Volume per 15 µl reaction
BioGX Xfree DNA Master Mix	1 cake	N/A
BioGX forward and reverse primers/probes	1 bead X µL rehydration volume	**x µL**
Purified Nucleic Acid Sample	N/A	**5 µL**
Molecular grade water	N/A	**15 µl - x µL - 5 µL**

g. Dispense prepared reaction mix **(10 µL for purified nucleic acid sample addition)** into PCR instrument compatible PCR tubes or plates.

h. Continue with step 3.

3. Add sample template

For Direct Sample Processing

a. Aliquot **30 µL** of **prepared reaction mix** (see **Table 1 and Table 3**) to each PCR tube/plate well you will use.

b. Add **20 µL** of **direct sample template** to each PCR tube/plate well containing dispensed, prepared reaction mix.

c. Seal PCR tubes/plates appropriately.

d. Quickly centrifuge to collect solutions at the bottom of the tube and to remove air bubbles.

For Purified Nucleic Acid Sample Processing

e. Aliquot **10 µL** of **prepared reaction mix** (see **Table 2 and Table 4**) to each PCR tube/plate well you will use.

f. Add **5 µL** of **purified nucleic acid sample template** to each PCR tube/plate well containing dispensed, prepared reaction mix.

g. Seal PCR tubes/plates appropriately.

h. Quickly centrifuge to collect solutions at the bottom of the tube and to remove air bubbles.

4. Set-up PCR cycling program

a. Program the appropriate PCR cycling protocol on the real-time PCR instrument of choice.

5. Run PCR

a. If the PCR tube/plate was stored before PCR, centrifuge briefly.

b. Place the plate in the real-time PCR instrument and start the cycling program.

ASSAY PERFORMANCE

BioGX Xfree DNA Master Mix is designed to detect 50 copies or less of the target nucleic acid per reaction when utilized in a properly optimized PCR.

REFERENCES

1. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
2. Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. Choosewood L.C. and Wilson D.E. (eds) (2009). HHS Publication No. (CDC) 21-1112.

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

Rev. #	Effective Date	Summary of Changes
02	05 APR 2024	Update workflow for direct sample processing.
01	19 NOV 2021	Initial Release.

SYMBOLS

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

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1500 First Avenue, North, L136, Birmingham, AL 35203, USA
Phone: +1.205.250.8055
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