



BioGX

Molecular Made Easy

Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri Open System PCR Reagents

REF 450-095-C-MAX

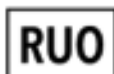
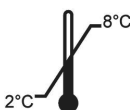


24 Reactions

Product Insert

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

For use with BD MAX™ System



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For Research Use Only

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

The Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri real-time PCR-based detection reagent is manufactured and packaged as an open system reagent (OSR) for use with open system platforms and has to be validated by the user. Examples of open system platforms are the BD MAX™ System (Windows® Software V4.72A or later).

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

PRODUCT OVERVIEW

The BioGX Sample-Ready™ OSR has been formulated in lyophilized format for the multiplex real-time PCR-based detection of DNA from Acanthamoeba spp. (18 rDNA gene¹), Balamuthia mandrillaris (18 rDNA gene²), Naegleria fowleri (18 rDNA gene¹) and a Sample Processing Control (SPC) (Drosophila DNA) for use with BD MAX extraction kit. The Drosophila DNA serves as both a sample processing control and an internal amplification control. One format for the lyophilized Sample-Ready OSR kits is available:

1. BD MAX™ System
REF 450-095-C-MAX

Note:

BD MAX™ System OSR (450-095-C-MAX) contains all PCR primers, probes, enzymes, dNTPs, MgCl₂, buffers, and other components required for PCR reaction. BD MAX extraction kits available from Becton, Dickinson and Company include a Drosophila DNA sequence which serves as a Sample Processing Control (SPC). No exogenous addition of SPC is needed.

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PACKAGE CONTENTS

BioGX REF: 450-095-C-MAX

Platform(s): BD MAX™ System

Each 24-reaction package contains two pouches:

1. First pouch contains 24 sealed BD MAX™ tubes of BioGX lyophilized Sample-Ready™ OSR, each tube sufficient for a 12.5 µL PCR reaction.
2. Second pouch contains 24 sealed BD MAX™ tubes, each containing 25 µL of OSR-specific BioGX Rehydration Buffer.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- Lyophilized Positive Control Template DNA Beads (10⁵ copies/bead)
 - BioGX Acanthamoeba spp. (Part number 720-0240)
 - BioGX Balamuthia mandrillaris (Part number 720-0241)
 - BioGX Naegleria fowleri (Part number 720-0242)
- BioGX Molecular Grade Water or equivalent
 - BioGX Rehydration Water (Part number: 800-0035-12)
- BD MAX™ ExK™ DNA-3 (US BD catalog no. 442821/ International BD catalog no. 442822)
- BD MAX™ PCR Cartridges (US and International BD catalog no. 437519)
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent
- Disposable nitrile gloves

WARNINGS AND PRECAUTIONS

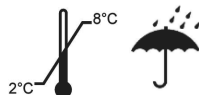


- For research use only. Not intended for human or animal diagnostics use.
- If handling biological samples, including used Extraction Kits and PCR Cartridges, treat as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29³ and in Biosafety in Microbiological and Biomedical Laboratories⁴.
- BioGX REF: 450-095-C-MAX has been quality control tested only with the BD Open System Extraction Kits on the BD MAXTM System.
- Do not use the reagents if the protective pouches are open or torn upon arrival.
- Close reagent protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing and store at 2-8 °C.
- Do not remove desiccant from the PCR master mix pouches.
- Do not use Sample-ReadyTM master mix if the desiccant is not present or is broken inside the Sample-ReadyTM master mix pouches.
- Do not use reagent tubes if the foil seal has been opened or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not use expired reagents and/or materials.



- Refer to BD MAXTM ExKTM DNA-3 Extraction Kit Instructions or to other respective nucleic acid extraction kits manufacturers' instructions for information about proper handling, cautions, and proper waste disposal.
- Do not mix septum caps between Sample Buffer Tubes or re-use septum caps as contamination may occur and compromise test results.
- Check BD Unitized Reagent Strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes).
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where samples or kits are being handled.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations.
- Use clean gloves when handling extraction kit components and PCR reagents and buffer tubes.

STORAGE REQUIREMENTS AND RECOMMENDATIONS



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

SAMPLE TYPES UTILIZED FOR QUALITY CONTROL TESTING

- Swab samples collected in Copan Universal Transport Media (UTM[®]), Copan ESwab[™]
- Cerebral Spinal Fluid (CSF)

REAGENT OPTICAL CONFIGURATION

Table 1. Optical Channel Configuration for REF 450-095-C-MAX.

Optical Channel (Fluorophore Equivalent)	Target
FAM	Acanthamoeba spp.
HEX	Naegleria fowleri
Texas Red	Balamuthia mandrillaris
Cy5	Unused
Cy5.5	SPC

QUALITY CONTROL AND EXTRACTION TESTING PARAMETERS**BD MAX™ System (BioGX REF: 450-095-C-MAX)**

As a starting point, users can import and install the Electronic User Defined Protocol (eUDP) that utilizes **ExK DNA-3** extraction kits onto the BD MAX™. BioGX's most current eUDP utilized for quality control of this product can be obtained by sending an email to TS@biogx.com. Please refer to the BD MAX™ user manual⁵ for uploading instructions.

SAMPLE VOLUMES UTILIZED DURING QUALITY CONTROL TESTING

The end user may choose to validate a different pretreatment method or volume of sample to load other than the sample processing used by BioGX for QC testing as outlined below.

Swab sample (3 mL Copan Universal Transport Media (UTM®))**BD MAX™ System**

Thoroughly vortex the sample prior to addition to the Sample Buffer Tube (SBT). Pipette 100 µL of sample into the SBT, aseptically place the BD™ septum cap on each SBT. Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.

Swab sample (1 mL Copan Universal Transport Media (UTM®) or Copan ESwab™)**BD MAX™ System**

Thoroughly vortex the sample prior to addition to the Sample Buffer Tube (SBT). Pipette 50 µL of sample into the SBT, aseptically place the BD™ septum cap on each SBT. Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.

Cerebral Spinal Fluid (CSF)**BD MAX™ System**

Thoroughly vortex the sample prior to addition to the Sample Buffer Tube (SBT). Pipette 200 µL of CSF sample into the SBT, aseptically place the BD™ septum cap on each SBT. Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.

BioGX QUALITY CONTROL TEST SETUP PROCEDURE

BD MAX™ System Quality Control Test Setup

Loading a Sample Buffer Tube (SBT)

1. Add the appropriate sample volume to each SBT.
2. Aseptically place BD™ septum cap on each SBT.
3. Vortex the SBT for 1-3 seconds.
4. Load the SBT into the extraction tray.

WEAR NITRILE GLOVES WHEN HANDLING LYOPHILIZED REAGENTS TO REDUCE THE GENERATION OF STATIC CHARGES. DO NOT USE LATEX GLOVES.

Assembly of BD MAX Extraction Strips and BioGX Reagents

1. Choose the appropriate BD MAX™ extraction kit (see above). DO NOT use BD MAX™ master mix or the blank 0.3 mL conical tubes that come with the extraction kit.
2. Load the selected extraction cartridges into the extraction tray, 1 per sample to be tested.
3. Snap one BD MAX™ ExK™ DNA Extraction tube into snap-in position 1 (Snap-1) of each extraction strip (**Figure 1**).
4. Snap one BioGX Sample-Ready™ lyophilized reagent tube into position 2 (Snap-2) of each extraction strip. Check to make sure the lyophilized cake is at the bottom of the tube prior to inserting into the strip. The funnel-shaped cake may be in any orientation (v, >, ^, <).
5. Snap one BioGX Rehydration Buffer tube into position 3 (Snap-3) of each extraction strip. Check to make sure the buffer is at the bottom of the tube prior to inserting into the strip. Position 4 (Snap-4) will remain empty.
6. Lift the tray and briefly examine the bottom of each strip to ensure all reagents are at the bottom of each tube and bubbles are not present in Extraction buffer reservoirs.
7. Proceed with worklist generation and sample loading per BD MAX™ operating instructions. Select the appropriate User Defined Protocol (UDP). Load the extraction tray and, if necessary, a new PCR card into the instrument, close the door, and click “Start Run.”
8. Analyze the results by opening the completed run file in the “Results” tab.

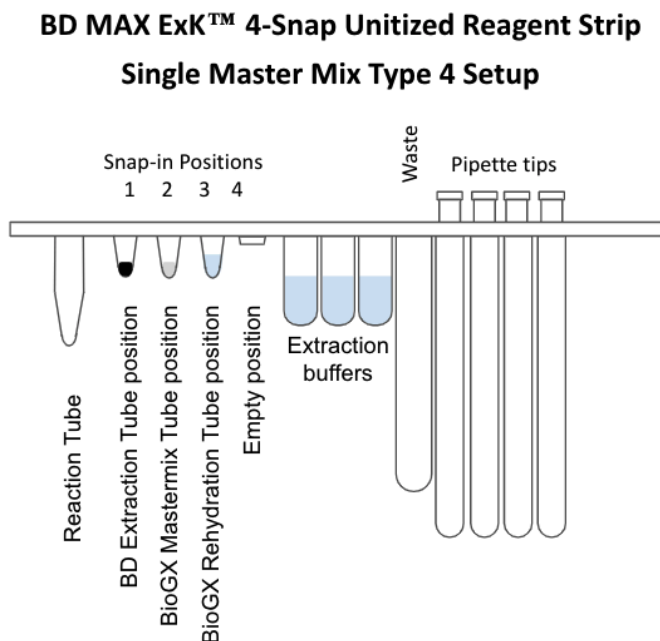


Figure 1. Diagram of BD MAX™ ExK™ 4-snap Unitized Reagent Strips.

Important Note

Always first insert all Snap-1 tubes, then all Snap-2 tubes, then all Snap-3 tubes. The Snap 4 position will remain empty unless the user has set up the reagent to run in dual master mix mode.

Approximately 25 µL of extracted nucleic acid remains in the position 3 tube after extraction. This may be removed and saved for further analyses after the run has been completed.

ASSAY PERFORMANCE

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

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INTERPRETATION OF RESULTS

Table 2. Multiplex PCR Results Interpretation for 450-095-C-MAX.

Acanthamoeba spp.	Naegleria fowleri	Balamuthia mandrillaris	SPC	Interpretation
+	-	-	+/-	Acanthamoeba spp. POSITIVE
-	+	-	+/-	Naegleria fowleri POSITIVE
-	-	+	+/-	Balamuthia mandrillaris POSITIVE
-	-	-	+	Acanthamoeba spp., Naegleria fowleri, Balamuthia mandrillaris NEGATIVE
-	-	-	-	Unresolved*

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









REFERENCES

1. Dobrowsky, Penelope H., et al. "Molecular detection of Acanthamoeba spp., Naegleria fowleri and Vermamoeba (Hartmannella) vermiformis as vectors for Legionella spp. in untreated and solar pasteurized harvested rainwater." Parasites & vectors 9.1 (2016): 1-13.
2. Onyango, Clayton O., et al. "Evaluation of a TaqMan array card for detection of central nervous system infections." Journal of clinical microbiology 55.7 (2017): 2035-2044.
3. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
4. 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.
5. BD MAX™ System User's Manual (refer to the latest revision) BD Life Sciences, Sparks, Maryland 21152 USA.

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

Rev. #	Effective Date	Summary of Changes
01	24 JAN 2025	Initial Release.

SYMBOLS

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



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