



BioGX

Molecular Made Easy

Acinetobacter baumannii, Klebsiella aerogenes/Enterobacter cloacae, Morganella morganii, Kingella kingae Open System PCR Reagents

REF 450-041-Series



24 Reactions 450-041-C-MAX

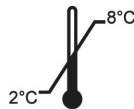


64 Reactions 450-041-LMP

Product Insert

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

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



RUO



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Acinetobacter baumannii, Klebsiella aerogenes/Enterobacter cloacae, Morganella morganii, Kingella kingae Open System PCR Reagents

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 Acinetobacter baumannii, Klebsiella aerogenes/Enterobacter cloacae, Morganella morganii, Kingella kingae 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), Applied Biosystems QuantStudio™ 5 (Design & Analysis software version 1.5.1 or later), and Bio-Rad CFX96 Touch™ (Maestro software version 1.1 or later) real-time PCR platforms.

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

PRODUCT OVERVIEW

The BioGX Sample-Ready™ OSR has been formulated in lyophilized format for the multiplex real-time PCR-based detection of DNA from Acinetobacter baumannii (ompA gene¹), Klebsiella aerogenes/Enterobacter cloacae (16S rRNA gene²), Morganella morganii (16S rRNA gene³), Kingella kingae (mdh gene⁴) 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. The configuration compatible with the ABI and Bio-Rad platform targets the human RNase P gene to serve as an endogenous extraction control. The two different formats for the lyophilized Sample-Ready OSR kits are available:

1. BD MAX™ System
REF 450-041-C-MAX
2. ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™ Platforms
REF 450-041-LMP

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Note:

BD MAX™ System OSR (450-041-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.

OSR for ABI and Bio-Rad Platforms (450-041-LMP) contain all PCR primers, probes, enzymes, dNTPs, MgCl₂, buffers, and other components required for the PCR reaction. No exogenous addition of SPC is needed.

PACKAGE CONTENTS

BioGX REF: 450-041-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.

BioGX REF: 450-041-LMP

Platform(s): ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™

Each 64-reaction package consists of one pouch:

The pouch contains 2 x 8-tube strips. Each tube contains BioGX lyophilized Sample-Ready™ OSR sufficient for 4 x 15 µL PCR reactions.

Note: 8-tube strips containing reagents **ARE NOT** compatible with ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™ instruments

Acinetobacter baumannii, Klebsiella aerogenes/Enterobacter cloacae, Morganella morganii, Kingella kingae Open System PCR Reagents

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- Lyophilized Positive Control Template DNA Beads (10^5 copies/bead)
 - BioGX Acinetobacter baumannii (Part number 720-0126)
 - BioGX Klebsiella aerogenes (formerly E. aerogenes)
(Part number 720-0127)
 - BioGX Enterobacter cloacae (Part number 720-0128)
 - BioGX Morganella morganii (Part number 720-0129)
 - BioGX Kingella kingae (Part number 720-0130)
- Lyophilized Positive Control Template DNA Beads (10^5 copies/bead) for ABI and Bio-Rad platforms
 - BioGX RNaseP (DNA) (Part number 720-0009)
- BioGX Molecular Grade Water or equivalent
 - BioGX Rehydration Water (Part number: 800-0035-12)
- BioGX Lyophilized Far Red Dye Calibration Kit for Applied Biosystems QuantStudio 5 (Part number 720-0219)
- 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
- Applied Biosystems QuantStudio 5 (0.2 mL) consumables
 - Thermo Fisher optical 8-tube strip (catalog no. 4316567)
 - Thermo Fisher ultra-clear optical caps, strips of 8 (catalog no. AB-0866)
 - Thermo Fisher 96-well optical clear reaction plates (catalog no. A36924)
 - Thermo Fisher 96-well qPCR plate seals (catalog no. AB-1170)
- Bio-Rad CFX96 Touch consumables
 - Bio-Rad 8-tube PCR strips without caps (catalog no. TLS0851)
 - Bio-Rad Optical flat 8-cap strips for PCR tubes (catalog no. TCS0803)
 - Bio-Rad 96-well plates (catalog no. HSP9655)
 - Bio-Rad 96-well plate sealing film, optical (catalog no. MSB1001)

Acinetobacter baumannii, Klebsiella aerogenes/Enterobacter cloacae, Morganella morganii, Kingella kingae Open System PCR Reagents

WARNINGS AND PRECAUTIONS

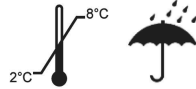


- For research use only. Not intended for human or animal diagnostics use.
- If handling biological samples, including used Extraction Kits and PCR Cartridges, treat as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29⁵ and in Biosafety in Microbiological and Biomedical Laboratories⁶.
- BioGX REF: 450-041-C-MAX has been quality control tested only with the BD Open System Extraction Kits on the BD MAXTM System.
- BioGX REF: 450-041-LMP has been quality control tested only with the Applied Biosystems QuantStudioTM 5 and Bio-Rad CFX96 TouchTM real-time PCR platforms.
- Do not use the reagents if the protective pouches are open or torn upon arrival.
- Close reagent protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing and store at 2-8 °C.
- Do not remove desiccant from the PCR master mix pouches.
- Do not use 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.



Acinetobacter baumannii, Klebsiella aerogenes/Enterobacter cloacae, Morganella morganii, Kingella kingae Open System 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 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

- Urine (neat or boric acid preserved)
- Swab samples collected in Copan Universal Transport Media (UTM[®]) or Copan ESwab[™]

REAGENT OPTICAL CONFIGURATION

Table 1A. Optical Channel Configuration for REF 450-041-C-MAX.

Optical Channel (Fluorophore Equivalent)	Target
FAM	Morganella morganii
HEX	Kingella kingae
Texas Red	Klebsiella aerogenes/Enterobacter cloacae
Cy5	Acinetobacter baumannii
Cy5.5	SPC

**Acinetobacter baumannii, Klebsiella
aerogenes/Enterobacter cloacae, Morganella
morganii, Kingella kingae
Open System PCR Reagents**

Table 1B. Optical Channel Configuration for REF 450-041-LMP^a.

Optical Channel (Fluorophore Equivalent)	Target
FAM	Morganella morganii
HEX	Kingella kingae
Texas Red	Klebsiella aerogenes/Enterobacter cloacae
Cy5	Acinetobacter baumannii
Cy5.5 ^a	RNase P

^aWhen using the Applied Biosystems Quantstudio 5 instrument, the BioGX Lyophilized Far Red Dye Calibration Kit for Applied Biosystems QuantStudio 5 (Part number 720-0219) will be needed to add this dye to the instrument dye library. Please refer to the Product Insert of 720-0219 available on www.biogx.com for instructions and further details¹⁰.

QUALITY CONTROL AND EXTRACTION TESTING PARAMETERS

BD MAXTM System (BioGX REF: 450-041-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 MAXTM. 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 MAXTM user manual⁷ for uploading instructions.

Acinetobacter baumannii, Klebsiella aerogenes/Enterobacter cloacae, Morganella morganii, Kingella kingae Open System PCR Reagents

ABI and Bio-Rad Platforms (BioGX REF: 450-041-LMP)

As a starting point, users can import and install a PCR run file onto:

1. Applied Biosystems QuantStudio™ 5 (Design & Analysis software version 1.5.1 or later)
2. Bio-Rad CFX96 Touch™ (Maestro software version 1.1 or later)

BioGX's most current ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™ PCR run files utilized for quality control of this product can be obtained by sending an email to TS@biogx.com. Please refer to the Applied Biosystems QuantStudio™5 user manual⁸ for uploading instructions. Please refer to the Bio-Rad CFX96 Touch™ user manual⁹ 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.

Urine collection (neat urine or boric acid preserved urine)

BD MAX™ System

Thoroughly vortex the sample prior to addition to the Sample Buffer Tube (SBT). Pipette 500 µL of neat urine or boric acid preserved urine 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.

ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™ (validated magnetic bead or silica column extraction kits)

Thoroughly vortex the sample prior to processing. Follow manufacturer recommendations for extraction of appropriate sample volume. Transfer 5 µL to purified nucleic acid to master mix as described in Section: *BioGX Quality Control Test Setup Procedure for ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™*.

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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 μ L 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.

ABI QuantStudio[™] 5 and Bio-Rad CFX96 Touch[™] (validated magnetic bead or silica column extraction kits)

Thoroughly vortex the sample prior to processing. Follow manufacturer recommendations for extraction of appropriate sample volume. Transfer 5 μ L to purified nucleic acid to master mix as described in Section: *BioGX Quality Control Test Setup Procedure for ABI QuantStudio[™] 5 and Bio-Rad CFX96 Touch[™]*.

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 μ L 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.

ABI QuantStudio[™] 5 and Bio-Rad CFX96 Touch[™] (validated magnetic bead or silica column extraction kits)

Thoroughly vortex the sample prior to processing. Follow manufacturer recommendations for extraction of appropriate sample volume. Transfer 5 μ L to purified nucleic acid to master mix as described in Section: *BioGX Quality Control Test Setup Procedure for ABI QuantStudio[™] 5 and Bio-Rad CFX96 Touch[™]*.

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.

**Acinetobacter baumannii, Klebsiella
aerogenes/Enterobacter cloacae, Morganella
morganii, Kingella kingae
Open System PCR Reagents**

**BD MAX ExK™ 4-Snap Unitized Reagent Strip
Single Master Mix Type 4 Setup**

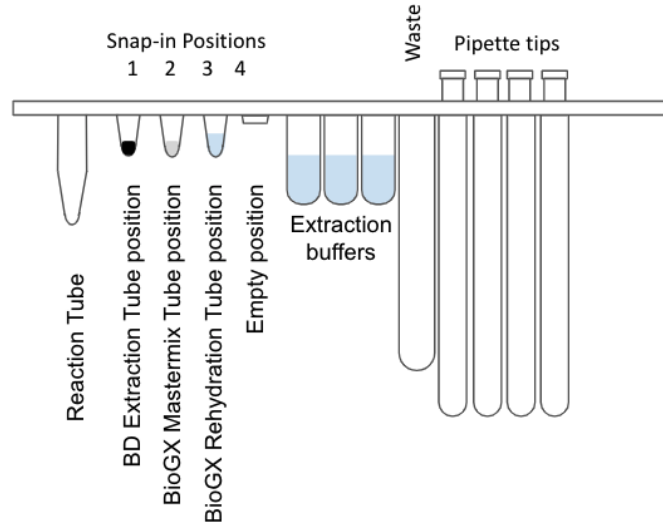


Figure 1. Diagram of BD MAX™ ExK™ 4-snap Unitized Reagent Strips.

Acinetobacter baumannii, Klebsiella aerogenes/Enterobacter cloacae, Morganella morganii, Kingella kingae Open System PCR Reagents

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.

ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™ Quality Control Test Setup

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

Assembly of BioGX Reagents

1. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
2. Transfer 40 µL of molecular grade water to one vial of lyophilized BioGX reagents. The rehydrated master mix is more than sufficient for 4 samples to be tested.

Note: 8-tube strips containing reagents **ARE NOT** compatible with ABI QuantStudio™ 5 and Bio-Rad CFX96 Touch™ instruments. Rehydrated master mix must be transferred to 8-tube PCR strips or 96-well PCR plates compatible with appropriate instruments.

3. Mix by gently pipetting up and down. (IMPORTANT: Keep rehydrated master mix in a cold block or on ice if setup cannot not be completed within 20 minutes. If the rehydrated master mix cannot be used immediately, it can be capped and stored up to 24 hours at 2-8°C, protected from light).
4. Transfer 10 µL of rehydrated master mix to the bottom of 4 empty wells.
5. To each well containing 10 µL of rehydrated master mix, add 5 µL of extracted sample.
6. Affix the appropriate optical caps or optical plate seals.
7. Pulse spin the sealed PCR plate or tube to mix and bring liquid to the bottom.
8. Load 8-tube PCR strips or 96-well PCR plates into the real-time PCR platform and start the run. Avoid unnecessary delay once tubes/plates are loaded into the real-time PCR instrument.

**Acinetobacter baumannii, Klebsiella
aerogenes/Enterobacter cloacae, Morganella
morganii, Kingella kingae
Open System PCR Reagents**

ASSAY PERFORMANCE

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

INTERPRETATION OF RESULTS

Table 2A. Multiplex PCR Results Interpretation for 450-041-C-MAX.

M. morganii	K. kingae	K. aerogenes/E. cloacae	A. baumannii	SPC	Interpretation
+	-	-	-	+/-	Morganella morganii POSITIVE
-	+	-	-	+/-	Kingella kingae POSITIVE
-	-	+	-	+/-	K. aerogenes/E. cloacae POSITIVE
-	-	-	+	+/-	Acinetobacter baumannii POSITIVE
-	-	-	-	+	Morganella morganii, Kingella kingae, K. aerogenes/E. cloacae, Acinetobacter baumannii NEGATIVE
-	-	-	-	-	Unresolved*

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

**Acinetobacter baumannii, Klebsiella
aerogenes/Enterobacter cloacae, Morganella
morganii, Kingella kingae
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Table 2B. Multiplex PCR Results Interpretation for 450-041-LMP.

M. morganii	K. kingae	K. aerogenes/E. cloacae	A. baumannii	RNase P	Interpretation
+	-	-	-	+/-	Morganella morganii POSITIVE
-	+	-	-	+/-	Kingella kingae POSITIVE
-	-	+	-	+/-	K. aerogenes/E. cloacae POSITIVE
-	-	-	+	+/-	Acinetobacter baumannii POSITIVE
-	-	-	-	+	Morganella morganii, Kingella kingae, K. aerogenes/E. cloacae, Acinetobacter baumannii NEGATIVE
-	-	-	-	-	Unresolved*

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

Acinetobacter baumannii, Klebsiella aerogenes/Enterobacter cloacae, Morganella morganii, Kingella kingae Open System PCR Reagents

REFERENCES









1. McConnell, Michael J., et al. "Quantitative real-time PCR for detection of Acinetobacter baumannii colonization in the hospital environment." *Journal of clinical microbiology* 50.4 (2012): 1412-1414.
2. Van Der Zee, Anneke, et al. "Molecular diagnosis of urinary tract infections by semi-quantitative detection of uropathogens in a routine clinical hospital setting." *PloS one* 11.3 (2016): e0150755.
3. Ferrario, C., et al. "Species-specific DNA probe and development of a quantitative PCR assay for the detection of Morganella morganii." *Letters in applied microbiology* 54.4 (2012): 292-298.
4. El Houmami, Nawal, et al. "A new highly sensitive and specific real-time PCR assay targeting the malate dehydrogenase gene of Kingella kingae and application to 201 pediatric clinical specimens." *Journal of clinical microbiology* 56.8 (2018).
5. Clinical and Laboratory Standards Institute. *Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).*
6. 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.*
7. *BD MAX™ System User's Manual (refer to the latest revision) BD Life Sciences, Sparks, Maryland 21152 USA.*
8. *QuantStudio™ Design and Analysis software User Guide, ThermoFisher Scientific, Waltham, Massachusetts, USA (Refer to the latest version).*
9. *CFX96 Touch, CFX96 Touch Deep Well, CFX Connect, and CFX384 Touch Systems Instruction Manual (refer to the latest version), Bio-Rad Laboratories, Inc., Hercules, California, USA (Refer to the latest version).*
10. *BioGX Lyophilized Far Red Dye Calibration Kit for Applied Biosystems QuantStudio™ 5 Real-Time PCR system Product number series 720-0219 (www.biogx.com)*
11. Tindall, B. J., G. Sutton, and G. M. Garrity. "Enterobacter aerogenes Hormaeche and Edwards 1960 (Approved Lists 1980) and Klebsiella mobilis Bascomb et al. 1971 (Approved Lists 1980) share the same nomenclatural type (ATCC 13048) on the Approved Lists and are homotypic synonyms, with consequences for the name Klebsiella mobilis Bascomb et al. 1971 (Approved Lists 1980)." *International journal of systematic and evolutionary microbiology* 67.2 (2017): 502-504.

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

Rev. #	Effective Date	Summary of Changes
03	22 FEB 2022	Update to include reference to BioGX Far Red Dye Calibration Kit for use with Applied Biosystems Quantstudio 5, Re-classification of Enterobacter aerogenes to Klebsiella aerogenes ¹¹
02	04 AUG 2021	Update branding, extraction control for ABI and Bio-Rad platforms and storage conditions.
01	28 MAY 2021	Initial Release.

**Acinetobacter baumannii, Klebsiella
aerogenes/Enterobacter cloacae, Morganella
morganii, Kingella kingae
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SYMBOLS

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



BioGX

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