BioGX
Molecular Made Easy

Xfree™ Mycoplasma pneumoniae Open System PCR Reagents

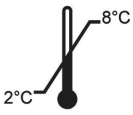
REF 450-093-E-PXL

Σ 16 Extraction-Free Direct Sample Tests

Σ 64 Extracted Sample Tests

Product Insert

For Research Use Only: Not intended for In Vitro Diagnostic Use
For use with ABI QuantStudio™ 5, Bio-Rad CFX96 Touch™, BioGX pixl.16 real-time PCR platform

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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 Xfree™ Mycoplasma pneumoniae 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 Applied Biosystems QuantStudio™ 5 (Design & Analysis software version 1.5.1 or later), Bio-Rad CFX96 Touch™ or BioGX pixl.16 (For Android® based software version 1.6.9 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 *Mycoplasma pneumoniae* (Mp181 - CARDS Toxin gene[1]), and RNase P to serve as an endogenous Sample Processing Control (SPC).

Note:

OSR 450-093-E-PXL contains all PCR primers, probes, enzymes, dNTPs, $MgCl_2$, buffers, and other components required for the PCR reaction. No exogenous addition of Sample Processing Control is needed.

PACKAGE CONTENTS

BioGX REF: 450-093-E-PXL
Platform(s): Applied Biosystems, Bio-Rad and BioGX pixl.16

Each 16-reaction/64-reaction package consists of two pouches:

1. The first pouch contains 2 x 8-tube PCR strips. Each tube contains BioGX lyophilized Sample-Ready™ reagents sufficient for 1 PCR reaction. Each pouch contains reagents for 16 PCR reactions when used in extraction-free format and for 64 PCR reactions when used in extracted format.

2. The second pouch contains 1 tube of Rehydration Buffer E (BioGX catalog no. 800-0031-E-L), each containing 650µL of Rehydration Buffer E.

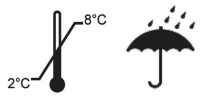
EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- Lyophilized Positive Control Template DNA Beads (10^5 copies/bead)
  - BioGX Mycoplasma pneumoniae (BioGX catalog no. 720-0010)
  - BioGX RNase P (DNA) (BioGX catalog no. 720-0009)
- 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)
- BioGX pixl™ Real-Time PCR Platform (pixl.16™) (BioGX catalog no. 650-016-PXL)
- BioGX pixl™ Real-Time PCR Platform (pixl.16™) instrument consumables (0.2 mL)
  - TempAssure® PCR 8-Tube Strips, Att. Optical Caps (BioGX catalog no. 010-280-ETS)
  - EasyStrip™ Plus Tube Strip with Attached Ultra Clear Caps (Thermo Fisher catalog no. AB2005) or
  - Axygen® 0.2 mL Polypropylene PCR Tube Strips and attached Flat Cap Strips, 8 Tubes/Strip, (Corning catalog no.: PCR-0208-AF-C)
- **Optional:**
  - BioGX pixl Barcode Scanner with Stand (BioGX catalog no. 650-726-SC-PXL)
- Calibrated micropipettes

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 M29[2] and in Biosafety in Microbiological and Biomedical Laboratories[3].
- BioGX REF: 450-093-E-PXL has been quality control tested only with the ABI QuantStudio™ 5, Bio-Rad CFX96 Touch™ and BioGX pixl.16 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-Ready™ master mix if the desiccant is not present or is broken inside the Sample-Ready™ 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.
- 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 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®), BD UVT, Viral Transport Media (VTM)[a]
-Nasal wash
-Pretreated sputum samples
-Pretreated bronchoalveolar lavage (BAL) samples

REAGENT OPTICAL CONFIGURATION

Table 1. Optical Channel Configuration for REF 450-093-E-PXL.

Optical Channel (Fluorophore Equivalent)	**Target**
FAM	Unused
HEX	Unused
Texas Red	Mycoplasma pneumoniae
Cy5	RNase P
Cy5.5	N/A

[a]*Centers for Disease Control. Preparation of Viral Transport Media (SOP#: DSR-052-05)*

QUALITY CONTROL AND TESTING PARAMETERS

ABI, Bio-Rad and BioGX pixl.16 Platforms

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)
3. BioGX pixl.16 real-time PCR platform (For Android® based software version 1.6.9 or later)

BioGX's most current ABI QuantStudio™ 5, Bio-Rad CFX96 Touch™ and BioGX pixl.16 PCR run files utilized for quality control of this product can be obtained by sending an email to TS@biogx.com. Please refer to the Applied Biosystems QuantStudio™ 5 user manual[4] for uploading instructions. Please refer to the Bio-Rad CFX96 Touch™ user manual[5] for uploading instructions. Please refer to the BioGX pixl.16 user manual[6] 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.

PCR SET-UP FOR DIRECT SAMPLES

Swab sample (3 mL Copan UTM®, BD UVT or VTM)

Thoroughly vortex the sample prior to processing. Transfer **20 µL** of direct sample to the rehydrated BioGX master mix as described in Section: *Direct Sample - Assembly of BioGX Reagents.*

Swab sample (1 mL Copan UTM®, BD UVT or VTM)

Thoroughly vortex the sample prior to processing. Transfer **20 µL** of direct sample to the rehydrated BioGX master mix as described in Section: *Direct Sample - Assembly of BioGX Reagents.*

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

Direct Sample - Assembly of BioGX Reagents

1. Prepare the appropriate number of 8-tube PCR strips or 96-well PCR plates.
2. Transfer **30 µL** of Rehydration Buffer E to one tube of lyophilized BioGX reagents. The rehydrated master mix is sufficient for 1 sample to be tested.

Note: 8-tube strips containing reagents are only compatible with the BioGX pixl.16 platform. 8-tube strips containing reagents ARE NOT compatible with Applied Biosystems or Bio-Rad Platforms. Rehydrated master mix must be transferred to 8-tube PCR tube strips or 96-well PCR plates compatible with appropriate instruments.

3. Mix by gently tapping the tube with your fingers until the lyophilized pellet has been dissolved.

(IMPORTANT: Keep rehydrated master mix in a cold block or on ice if setup cannot be completed within 20 minutes. If the rehydrated master mix cannot be used immediately, it can be capped and stored up to 24 hours at 2-8°C, protected from light).

4. To each PCR tube containing **30 µL** of rehydrated master mix, add **20 µL** of direct sample.
5. Affix the optical caps/seals to the PCR tube strips/plates.
6. Pulse spin the sealed PCR tube strips/plates.

7a. **For BioGX pixl.16 Platform:** Load PCR tube strips into the real-time PCR platform and start the run. Avoid unnecessary delay once samples are loaded into the real-time PCR instrument.

7b. **For Applied Biosystems and Bio-Rad Platforms:** Transfer rehydrated master mix to 8-tube PCR tube strips or 96-well PCR plates compatible with the appropriate instrument and start the run. Avoid unnecessary delay once samples are loaded into the real-time PCR instrument.

PCR SET-UP FOR EXTRACTED SAMPLES

Swab sample (3 mL Copan UTM®, BD UVT or VTM)

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 of purified nucleic acid to master mix as described in Section: *Extracted Sample - Assembly of BioGX Reagents.*

Swab sample (1 mL Copan UTM®, BD UVT or VTM)

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 of purified nucleic acid to master mix as described in Section: *Extracted Sample - Assembly of BioGX Reagents.*

Nasal Wash

Pretreatment: Freeze-thaw of nasal wash samples can provide better extraction results and reduce inhibitory effects.

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 of purified nucleic acid to master mix as described in Section: *Extracted Sample - Assembly of BioGX Reagents.*

Pretreated sputum and pretreated bronchoalveolar lavage (BAL) samples

PRETREATMENT PROCEDURES FOR VISCOUS SAMPLES TYPES (i.e., sputum and BAL)

APPROPRIATE LOCKING-CAP TUBES OR A LID-LOCK RACK MUST BE USED WHEN SAMPLES ARE BOILED. THE END USER SHOULD USE APPROPRIATE BIOSAFETY PROTOCOLS (INCLUDING A BIOSAFETY HOOD AND RESPIRATOR) WHEN PROCESSING SPUTUM or BAL SAMPLES THAT POTENTIALLY CONTAIN *MYCOBACTERIA*.

For viscous samples, the use of a mucolytic agent to reduce viscosity and support efficient DNA extraction is recommended. Examples of three different pretreatment protocols to reduce viscosity of sputum or BAL samples are outlined below. Note: Pretreatment reagents are not included with BioGX 450-093-E-PXL.

1. **Proteinase K Pretreatment:** Pellet sample (250 µL sputum or 500 µL BAL) by centrifugation at 20,000 x g for 15 min, decant and wash pellet with 1 mL 20mM Tris HCl pH 8, centrifuge at 20,000 x g for 15 min, decant and add 250 µL of Proteinase K solution (1 mg/mL), incubate at 56°- 65°C for 30 minutes. After Proteinase K digestion, heat to 100°C for 10-15 minutes. Allow for cooling to room temperature.

   BioGX pixl.16 Platform (validated magnetic bead or silica column extraction kits)
   Once the pretreated sample has cooled to ambient temperature, follow manufacturer recommendations for extraction of appropriate sample volume of pretreated sample into validated magnetic-bead or silica column extraction device and proceed with nucleic acid purification as described in Section: *Extracted Sample - Assembly of BioGX Reagents.*

2. **Copan SL solution Pretreatment:** (Copan catalog #099CE.A) is a mucolytic agent that supports rapid digestion but does not provide decontamination of natural flora. Manufacturer recommendations should be followed. Depending on the mucopolysaccharide content of the sample, incubation time can range from 15 minutes to 120 minutes.

   Validated magnetic bead or silica column extraction kits
   Once the pretreated sample has cooled to ambient temperature, follow manufacturer recommendations for extraction of appropriate sample volume of pretreated sample into validated magnetic-bead or silica column extraction device and proceed with nucleic acid purification as described in Section: *Extracted Sample - Assembly of BioGX Reagents.*

3. **BD BBL® MycoPrep™ Pretreatment:** (BD catalog # 240862) supports both mucolytic digestion and decontamination of natural flora. Manufacturer recommendations for BD BBL® MycoPrep™ should be followed. Depending on the mucopolysaccharide content of the sample, incubation time can range from 15 minutes to 30 minutes.

Validated magnetic bead or silica column extraction kits

Once the pretreated sample has cooled to ambient temperature, follow manufacturer recommendations for extraction of appropriate sample volume of pretreated sample into validated magnetic-bead or silica column extraction device and proceed with nucleic acid purification as described in Section: *Extracted Sample - Assembly of BioGX Reagents.*

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

Extracted Sample - 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 tube of lyophilized BioGX reagents. The rehydrated master mix is more than sufficient for 4 samples to be tested.

Note: Rehydrated master mix must be transferred to clean 8-tube PCR strips or 96-well PCR plates compatible with appropriate Applied Biosystems, Bio-Rad and BioGX pixl.16 platform.

3. Mix by gently pipetting up and down.

(IMPORTANT: Keep rehydrated master mix in a cold block or on ice if setup cannot be completed within 20 minutes. If the rehydrated master mix cannot be used immediately, it can be capped and stored up to 24 hours at 2-8°C, protected from light).

4. Transfer **10 µL** of rehydrated master mix to the bottom of **4 clean, 8-tube PCR strips or 96-well PCR plates compatible with appropriate Applied Biosystems, Bio-Rad and BioGX pixl.16 platform.**
5. To each tube containing **10 µL** of rehydrated master mix, add **5 µL** of extracted sample.
5. Affix the optical caps/seals to the PCR tube strips/plates.
6. Pulse spin the sealed PCR tube strips/plates.
7. Load PCR tube strips/plates into the real-time PCR platform and start the run. Avoid unnecessary delay once samples are loaded into the real-time PCR instrument.

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 2. Multiplex PCR Results Interpretation for 450-093-E-PXL.

Mycoplasma pneumoniae	RNase P	Interpretation
+	+/-	Mycoplasma pneumoniae POSITIVE
-	+	Mycoplasma pneumoniae NEGATIVE
-	-	Unresolved*

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

REFERENCES

1. Thurman, Kathleen A., et al. "Detection of Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella spp. in clinical samples using a single-tube multiplex real-time PCR assay." Diagnostic microbiology and infectious disease 70.1 (2011): 1-9.
2. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
3. Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. (2009). Choosewood L.C. and Wilson D.E. (eds). HHS Publication No. (CDC) 21-1112.
4. QuantStudio™ Design and Analysis software User Guide, ThermoFisher Scientific, Waltham, Massachusetts, USA (Refer to the latest version).
5. 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).
6. pixl™ Real-Time PCR Platform Instructions for Use Manual (refer to the latest version).

REVISION HISTORY

Revision	Date	Description of Change
02	06 MAR 2024	Update to recommend direct and extracted sample workflow with BioGX pixl.16, Applied Biosystems Quantstudio™ 5, Bio-Rad CFX96 Touch™ platforms.
01	28 FEB 2023	Initial Release

SYMBOLS

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

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