BioGX
Molecular Made Easy

Mycoplasma pneumoniae, Legionella spp., Chlamydia pneumoniae, Chlamydia psittaci OSR for BD MAX™

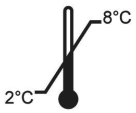
REF **350-068-C-MAX**

Σ 24 Reactions

Product Insert

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

For use with BD MAX™ System



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FOR LABORATORY USE ONLY

This product is manufactured and packaged as an Open System Reagent for the BD MAX™ system. It is the responsibility of the end user to determine the analytical performance of the reagents in an appropriately designed validation study. BioGX makes no claims regarding the clinical sensitivity and specificity of these reagents.

PLEASE READ ENTIRE INSERT BEFORE PROCEEDING WITH TEST SETUP.

This information is for use with BD MAX™ Windows® software release V4.70A or later.

Product Overview

This package contains one BioGX Sample-Ready™ kit for the multiplex detection of DNA from *Mycoplasma pneumoniae* (Mp181 - CARDS Toxin gene[1]), *Legionella* species (*L. pneumophila, L. longbeachae- ssr*A gene[1]), *Chlamydia pneumoniae* (*arg*R gene[1]), *Chlamydia psittaci (omp*A gene[2]) and a *Drosophila* sample processing control (SPC). The SPC in the multiplex targets the *Drosophila* control template present in the extraction reagents of the BD MAX™ ExK™ DNA series extraction kits, so no external addition of SPC is required. The SPC serves as both a sample extraction control and an internal amplification control (IAC). Each tube of Sample-Ready™ lyophilized reagents contains all PCR primers, probes, enzyme, dNTPs, $MgCl_2$, buffers, and other components required for analysis of one sample. BD MAX™ PCR Master Mix is not required.

BioGX has optimized this product for full extraction mode use on the BD MAX™ platform with BD ExK™ DNA-1 Open System extraction kits.

Extraction Kit ExK DNA-1 REF 442818

Basic suggested extraction processing parameters for **Nasal wash, pretreated sputum, pretreated bronchoalveolar lavage (BAL)** samples have been provided below.

Package Contents

Each 24-reaction package contains two pouches:

The first pouch contains 24 tubes of BioGX lyophilized reagents for detection of DNA from *Mycoplasma pneumoniae*, *Legionella* species, *Chlamydia pneumoniae, Chlamydia psittaci.* Each tube is sufficient for a 12.5 µL reaction, sealed in BD MAX™ 0.3 mL conical tubes.

The second pouch contains 24 tubes, each containing 25 µL of BioGX Rehydration Buffer sealed in BD MAX™ 0.3 mL conical tubes.

Not included but available through BioGX

Lyophilized Positive Control Template RNA Beads (10^5 copies/bead)

Mycoplasma pneumoniae	Part number 720-0010
Legionella pneumophila	Part number 720-0011
Legionella longbeachae	Part number 720-0150
Chlamydia pneumoniae	Part number 720-0012
Chlamydia psittaci	Part number 720-0149

WARNINGS AND PRECAUTIONS

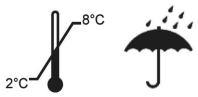
- For laboratory use only.
- Treat all biological samples, including used Extraction Kits and PCR Cartridges, as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29[3] and in Biosafety in Microbiological and Biomedical Laboratories[4].
- This test has been optimized only with the BD Open System Extraction Kits and sample types listed in this procedure. The performance of this assay with other sample 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 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.

- Each Sample-Ready™ Master Mix and BioGX Rehydration Buffer tube is used to process a single sample. Do not reuse Sample-Ready™ Master Mix or BioGX Rehydration Buffer tubes.

- Refer to BD MAX™ ExK™ DNA-1 Extraction Kit 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 Laboratory 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. Tightly reseal the pouch with unused reactions and immediately return to storage at 2-8°C after opening. To mitigate reagent performance degradation from exposure to moisture, BioGX suggests using the entire contents of the opened pouch within 2 months; however, the user may choose to verify an extended working time (>2 months) by performance testing with positive controls and an examination of the sample preparation control target.

Choose a BD MAX™ ExK™ Series Extraction Kit Appropriate for Your Sample Type

BioGX recommends using the BD MAX™ ExK™ DNA-1 extraction kit with this product.

Install a User Defined Protocol on the BD MAX™

Windows® Software V4.70A or later:

It will be necessary to import an Electronic User Defined Protocol (eUDP) onto the BD MAX™. The most current eUDP 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 "Int. Product Documents". Choose the appropriate product number under "Instructions for Use Manual & Product Inserts" and download the eUDP. Please refer to the BD MAX™ user manual[5] for uploading instructions.

To manually install a protocol the basic parameters below are suggested:

Extraction Type

BioGX recommends the following extraction for the sample types listed below:

BD MAX™ ExK™ DNA-1 (Urine) for use with nasal wash, pretreated sputum, and pretreated bronchoalveolar lavage (BAL).

NOTE: When manually setting up eUDPs the correct snap configuration must be selected to match 4-snap extraction strip type. Ensure the extraction type selected on the drop down indicates "4-snap".

Master Mix Format

Use Type 4 workflow protocol.

Sample Extraction Parameters

It is suggested that the user set up different protocol(s) for the various sample types to be tested. The BD MAX™ software allows one to copy an existing protocol, change specific parameters, and save a new protocol under a different name.

When programming the Sample Extraction Parameters one must allow for a 250 µL "dead volume" of liquid in the Sample Buffer Tube (SBT) that cannot be pipetted out of the tube or processed by the BD MAX™.

If the Sample Extraction Parameters differ from the default settings for the extraction kit being used, it will be necessary to program user defined parameters. The following parameters are suggested as a basic protocol for processing of the samples listed below:

BD MAX™ ExK™ DNA-1 (Urine) - Nasal wash, pretreated sputum and pretreated bronchoalveolar lavage (BAL)

Sample Lysis Time	20 minutes
Sample Lysis Temperature	62°C
Sample Volume	700 µL
Leave all other settings at default values	

Please refer to the "Sample Pretreatment and SBT Loading Volume" section below.

The end user may also define a different custom protocol by following the guidelines included in the BD ExK™ extraction kit product insert.

PCR Cycling Conditions

Cycling Stage 1: Hold 99°C for 300 sec

Cycling Stage 2: Three Temperatures x 40 Cycles of

99°C for 10 sec, Optics Off
62°C for 25 sec, Optics On
72°C for 13.7 sec, Optics Off

Channel Settings

475/520 channel	*Chlamydia psittaci*	set Gain to 60	set Threshold to 200
530/565 channel	*Chlamydia pneumoniae*	set Gain to 60	set Threshold to 200
585/630 channel	*Legionella* species	set Gain to 60	set Threshold to 200
630/665 channel	*Mycoplasma pneumoniae*	set Gain to 60	set Threshold to 200
680/715 channel	Sample Processing Control	set Gain to 60	set Threshold to 200

Set melt gain in all channels to 0.

BioGX suggests the minimum threshold settings listed above for each channel as a starting point for validation, but the final settings must be determined by the end user during and after laboratory validation with appropriate controls.

Ct Calculation

Select option to “Call Ct at Threshold Crossing”.

Setting the Ct min and max is optional depending upon requirements of the end user. The end user may select the valid Ct range for each target if desired.

Color Compensation

BioGX suggests the following initial color compensation settings:

1. Excitation Channel 475/520 with False Receiving Channel 530/565 – Set to "1"
2. Excitation Channel 530/565 with False Receiving Channel 475/520 – Set to "2.5"
3. Excitation Channel 530/565 with False Receiving Channel 585/630 – Set to "1"
4. Excitation Channel 585/630 with False Receiving Channel 630/665 – Set to "3"
5. Excitation Channel 630/665 with False Receiving Channel 585/630 – Set to "4"

The final settings must be determined by the end user during and after laboratory validation with appropriate controls.

BioGX suggests the minimum threshold and color compensation settings listed above for each channel as a starting point for validation, but the final settings must be determined by the end user during and after laboratory validation with appropriate controls.

Melt Settings

Melt settings are not needed for BioGX reagents.

Result Logic

To simplify reports, result logic may be used as appropriate for each target.

Sample Pretreatment and SBT Loading Volume

The end user may choose to validate a different pretreatment method or volume of sample to load other than the suggested sample processing outlined below. If different sample volumes are deemed appropriate for the existing workflow of the laboratory it will be necessary to modify Guardrail parameters accordingly.

Nasal wash samples

Pretreatment: Freeze-thaw of nasal wash samples can provide better extraction results and reduce inhibitory effects.
Pipette **700 µL** of Viral Transport Medium (eg. BD Universal Viral Transport or Copan Universal Transport Medium) and **50 µL** of *pretreated* nasal wash sample into the Sample Buffer Tube (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. Thoroughly vortex the sample prior to addition to the SBT.

Sputum and BAL samples

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 350-068-C-MAX.

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. **Once at ambient temperature, pipette 200 µL of sample into the Sample Buffer Tube (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.**

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. **Pipette 200 µL of sample into the Sample Buffer Tube (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.**

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. **Pipette 200 µL of sample into the Sample Buffer Tube (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.**

Additional treatment for samples showing inhibition after pretreatment

Add 40 µL of pretreated sputum or BAL to 160 µL of water to dilute. Then add 25 µL of Proteinase K solution (1 mg/mL), briefly mix with a vortex mixer, and incubate at 60°C for 30 minutes. After Proteinase K digestion, the sample should be heated to 100°C for 30 minutes. **Once at ambient temperature, pipette 200 µL of sample into the Sample Buffer Tube (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.**

Do not perform sample pre-treatment in the BD MAX Sample Buffer Tubes.

Other Sample Types

Please contact BioGX for processing suggestions if collecting sample types other than those described above, as some sample types and/or transport media can be inhibitory to PCR or disrupt extraction without appropriate Guardrail and processing volume adjustments.

General Sample Rerun Strategy

The end user should determine the appropriate strategy for available sample volume and laboratory workflow.
With hard to obtain samples, the end user may desire a processing strategy that allows the remaining sample in the Sample Buffer Tube (SBT) to be rerun. If so, the Sample Volume in the Guardrail setting should be reduced accordingly.

General Instructions for 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.

Instructions for Using BioGX Sample-Ready™ Reagents on the BD MAX™

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

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 position 1 (Snap-1) of each extraction strip (Figure 1).
4. Snap one BioGX Sample-Read™ 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.

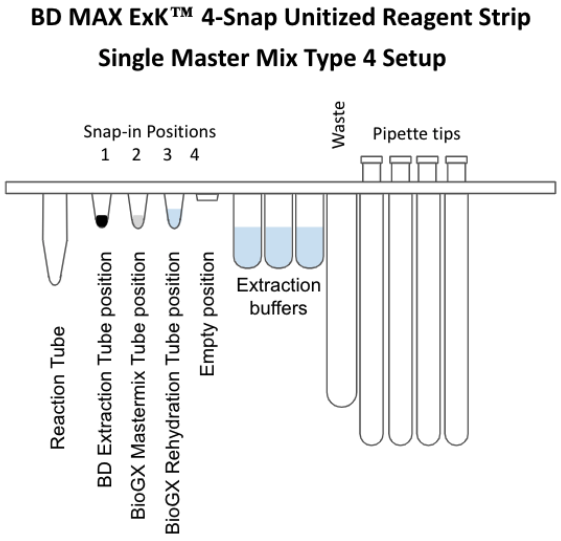


Figure 1 - Diagram of BD MAX™ ExK™ 4-snap Unitized Reagent Strips

6. Lift the tray and briefly examine the bottom of each strip to ensure all reagents are at the bottom of each tube.
7. Proceed with worklist generation and sample loading per BD MAX™ operating instructions. Select the appropriate User Defined Protocol (UDP).
8. Load the extraction tray and, if necessary, a new PCR card into the instrument, close the door, and click "Start Run."
9. Analyze the results by opening the completed run file in the "Results" tab.

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 DNA remains in the position 3 tube after extraction. This may be removed and saved for further analyses after the run has completed.

Chlamydia psittaci Clade and Cross-reactivity

For samples that are reported in the *Chlamydia psittaci* optical channel, cross-reactivity with the following *Chlamydia* species cannot be ruled out. This assay is not designed for species differentiation of the closely related *Chlamydia psittaci* clade species[6-8] (see list below). Human infection with the above listed organisms is relatively uncommon, but has been reported in the literature[9-11].

Chlamydia psittaci clade species

- *Chlamydia abortus*
- *Chlamydia buteonis*
- *Chlamydia caviae*
- *Chlamydophila felis*

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. Branley, J. M., et al. "Real-time PCR detection and quantitation of Chlamydophila psittaci in human and avian samples from a veterinary clinic cluster." European Journal of Clinical Microbiology & Infectious Diseases 27.4 (2008): 269-273.
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.
6. Longbottom, David, et al. "Whole genome de novo sequencing and comparative genomic analyses suggests that Chlamydia psittaci strain 84/2334 should be reclassified as Chlamydia abortus species." BMC genomics 22.1 (2021): 1-18.
7. Everett, Karin DE, Robin M. Bush, and Arthur A. Andersen. "Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms." International Journal of Systematic and Evolutionary Microbiology 49.2 (1999): 415-440.
8. Sachse, Konrad, et al. "Emendation of the family Chlamydiaceae: proposal of a single genus, Chlamydia, to include all currently recognized species." Systematic and applied microbiology 38.2 (2015): 99-103.
9. Lutz-Wohlgroth, Leslie, et al. "Chlamydiales in guinea-pigs and their zoonotic potential." Journal of Veterinary Medicine Series A 53.4 (2006): 185-193.
10. Heddema, Edou R., et al. "Typing of Chlamydia psittaci to monitor epidemiology of psittacosis and aid disease control in the Netherlands, 2008 to 2013." Eurosurveillance 20.5 (2015): 21026.
11. Ramakers, Bart P., et al. "Zoonotic Chlamydia caviae presenting as community-acquired pneumonia." New England Journal of Medicine 377.10 (2017): 992-994.

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

Rev. #	Effective Date	Summary of Changes
05	30 SEP 2022	Addition of cross-reactivity statement for C. psittaci primer and probe set
04	21 OCT 2021	Correction of incorrect name on title page from 350-068-A-MAX to 350-068-C-MAX
03	27 AUG 2021	Update branding and shipment temperature.
02	05 MAY 2021	Update Figure 1, update references, update website pathway, edit pretreatment procedure.
01	03 MAR 2020	Initial Release.

SYMBOLS

Symbol	Meaning	Symbol	Meaning
	Catalog number		Contains sufficient for <n> tests
	Do not reuse		Manufacturer
	Keep dry		Temperature limitation
	Consult instructions for use		Biological Risks

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