



# ***Pneumocystis jirovecii* Open System Reagents for BD MAX™**

**REF 350-010-A-MAX**

## **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 REAGENT SETUP.**

**This information is for use with BD MAX™ Windows™ software release V4.32A or later.**

#### **Product Overview**

This package contains one BioGX Sample-Ready™ kit for the multiplex detection of DNA from *Pneumocystis jirovecii* (*msg*), 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™ 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<sub>2</sub>, 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 against panels of control strains, near-neighbors, and unrelated organisms.

Basic suggested extraction processing parameters for Pretreated Sputum samples/specimens have been provided below.

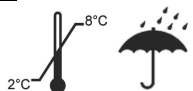
#### **Package Contents**

Each 24-reaction package contains two pouches:

The first pouch contains 24 tubes of BioGX lyophilized reagents for *Pneumocystis jirovecii*, each sufficient for a 12.5 µL reaction, sealed in BD MAX™ 0.3 mL conical tubes.

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

#### **Storage Requirements and Recommendations**



Product ships at ambient temperature, but BioGX recommends long-term storage at 2-8°C. Reagents have been examined 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

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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 2 weeks; however, the user may choose to verify an extended working time (>2 weeks) by performance analysis 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™**

It will be necessary to manually input or import a User Defined Protocol (UDP) on the BD MAX™. To import a basic UDP, please contact support@biogx.com for the necessary file. Please refer to the BD MAX™ user manual for uploading instructions. **To manually install a protocol the basic parameters below are suggested:**

### **Extraction Type**

BD MAX™ ExK™ DNA-1 Urine extraction protocol.

### **Master Mix Format**

Windows™ Software V4.32 A or later:

Use Type 4 workflow protocol.

### **Guardrail (Parameters for Extraction)**

It is suggested that the user set up different protocols for the various sample types to be analyzed. 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 guardrail 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 Guardrail 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:

### **Pretreated Sputum**

|  |            |
|--|------------|
| 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/Specimen 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™ DNA-1 extraction kit product insert.

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## **PCR Cycling Conditions**

Cycling Stage 1: Hold 99°C for 120 sec

Cycling Stage 2: Two Temperatures x 45 Cycles of

99°C for 12 sec, Optics Off  
62°C for 46.7 sec, Optics On

## **Channel Settings**

|                 |                                     |                |                      |
|-----------------|-------------------------------------|----------------|----------------------|
| 475/520 channel | <i>Pneumocystis jirovecii</i> (msg) | set Gain to 60 | set Threshold to 100 |
| 530/565 channel | Unused                              | set Gain to 0  |                      |
| 585/630 channel | Unused                              | set Gain to 0  |                      |
| 630/665 channel | Unused                              | set Gain to 0  |                      |
| 680/715 channel | Sample Processing Control           | set Gain to 60 | set Threshold to 100 |

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.

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## **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 the each target if desired.

## **Result Logic**

To simplify reports, set result logic as appropriate for each target.

## **Sample/Specimen Pretreatment and SBT Loading Volume**

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



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## **Sputum Processing**

**Pretreatment:** It is suggested that sputum be pretreated with a NALC-NaOH digestion/decontamination step followed by a proteinase K digestion and boiling treatment. For laboratories not currently processing sputum, the user may consider using the BD BBL® MycoPrep™ kit, a commercially available specimen digestion/decontamination kit for processing of Mycobacterial specimens (<http://www.bd.com/ds/technicalCenter/clsi/clsi-MYCOPREP.pdf>). Many variations of Proteinase K treatment may work. One strategy is to add 250 µL of 1 mg/mL ProK solution to the resuspended pellet obtained after NALC-NaOH treatment and neutralization, and incubate at 56° - 65°C for 30 minutes. After ProK digestion, the sample should be heated to 100°C for 10-15 minutes (using an appropriate tube).

**SBT Loading:** It is suggested to add **200 µL** of pretreated sputum sample into the SBT (please refer to “Sputum Processing Notes” section below).

### **Sputum Processing Notes:**

Pre-processing of sputum samples with NALC-NaOH and PBS neutralization, followed by a 30 minute digestion with Proteinase-K, and boiling for 10-15 minutes, has been validated to work well with extraction using BD MAX™ ExK™ DNA-1 kits. Up to a 750 µL sample of sputum pretreated in this manner may be loaded into the ExK™ DNA-1 Sample Buffer Tube (SBT) with appropriate Guardrail changes. The most reliable results (lacking PCR inhibition) may be obtained for a loading of 200 µL of pre-treated sample as suggested above, but processing of sputum can produce pellets that differ significantly from specimen to specimen.

For extraction purposes, the Proteinase-K and boiling treatments may only prove to be necessary for extremely turbid samples, but they offer additional benefits in terms of inactivation of live organisms. The end user may choose to validate other laboratory-appropriate pretreatment methods or commercial kits for NALC-NaOH treatment.

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

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 SAMPLES THAT POTENTIALLY CONTAIN *MYCOBACTERIA*.

Reference of Interest for pretreatment respiratory specimens for extraction on the BD MAX™:  
[http://www.pamm.nl/fileadmin/mediaarchive/corporate/bestanden/Poster\\_EMMD\\_Scheveningen\\_041013\\_BD\\_Max.pdf](http://www.pamm.nl/fileadmin/mediaarchive/corporate/bestanden/Poster_EMMD_Scheveningen_041013_BD_Max.pdf)

## **General Sample Rerun Strategy**

In the unlikely event of a run failure with hard to obtain specimens, 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. The end user should determine the appropriate strategy for available sample volume and laboratory workflow.

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## **General Instructions for Loading a Sample Buffer Tube (SBT)**

1. Add the appropriate sample/specimen 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™ mastermix or the blank 0.3 mL conical tubes that come with the extraction kit.
2. Load the selected extraction cartridges into extraction tray, 1 per specimen to be analyzed.
3. Snap one BD MAX™ ExK™ DNA Extraction tube into position 1 (Snap 1) of each extraction strip.
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 bottom of tube prior to inserting into 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 bottom of tube prior to inserting into strip.
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 as defined in the “Channel Settings” section.

## **Important Note**

Always first insert all Snap 1 tubes, then all Snap 2 tubes, then all Snap 3 tubes.

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.

## **Target Detection and Cross-Reactivity**

Cross-reactivity studies were performed with positive and negative specimens inoculated with bacterial, viral, or fungal organisms to final concentrations known to produce robust real-time PCR amplification when run with gene specific primers. Assay-specific target organisms or genes listed below were amplified/detected over a wide range of concentrations. None of the following non assay-specific organisms reacted with *Pneumocystis jirovecii* primer pairs and probes for BD MAX™.

*Bordetella pertussis*, *Bordetella parapertussis*, *Mycobacterium tuberculosis*, *Legionella* spp. (various), *Mycoplasma pneumoniae*, *Chlamydomonas pneumoniae*, Adenovirus, Rotavirus, Astrovirus, Enterovirus, HSV-1, HSV-2, VZV, *Campylobacter urealyticus*, *Campylobacter lari*, *Campylobacter upsaliensis*, *Campylobacter hyointestinalis*, *Campylobacter concisus*, *Coccidioides immitus*, Parechovirus, *Listeria* spp. (various), *Salmonella* spp. (various), *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *E. coli* spp. (various), Influenza A, Influenza B, RSV, *Burkholderia cepacia*, *Achromobacter* spp. (various), *Candida glabrata*, *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Trichomonas vaginalis*, *Shigella* spp. (various), *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*

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*\*\*Quantitative PCR complete master mix containing GoTaq® Hot Start Polymerase manufactured by Promega Corporation for distribution by BioGX, Inc. Licensed to Promega under U.S. Patent Nos. 5,338,671 and 5,587,287 and their corresponding foreign patents. For Research Use Only. Not for any clinical or therapeutic use in humans or animals.*

**Please call BioGX, or email [support@biogx.com](mailto:support@biogx.com) with any questions you may have regarding this product.**

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