



Pan-Aspergillus

Open System Reagents for BD MAX™

REF 350-058-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 TEST SETUP.

This information is for use with BD MAX™ Windows™ software release V4.72A or later.

Product Overview

This package contains one BioGX Sample-Ready™ kit for the multiplex detection of DNA from various species of *Aspergillus* (rRNA gene), 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, MgCl₂, 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: Bronchoalveolar lavage (BAL) and sputum.

Package Contents

Each 24-reaction package contains two pouches:

The first pouch contains 24 tubes of BioGX lyophilized reagents for CMV each 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 DNA Beads (10⁵ copies/bead)

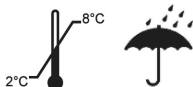
-Pan-Aspergillus - Part number 720-0118

Pan-Aspergillus

Open System Reagents for BD MAX™

REF 350-058-A-MAX

Storage Requirements and Recommendations



Product ships at ambient temperature, 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 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 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 kits with this product.

Install a User Defined Protocol on the BD MAX™

Windows™ Software V4.72A or later:

It will be necessary to manually input or import a User Defined Protocol (UDP) onto the BD MAX™. To import a basic UDP, please refer to our website at www.biogx.com/eu or contact eu@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) for use with BAL and sputum samples.

Master Mix Format

Use Type 4 workflow protocol.

Sample Extraction Parameters

It is suggested that the user set up different protocols 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™.

Pan-Aspergillus

Open System Reagents for BD MAX™

REF 350-058-A-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) - BAL and sputum samples

Default Sample Lysis Time	9 minutes
Default Sample Lysis Temperature	62°C
Default Sample Volume	500 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™ extraction kit product insert.

PCR Cycling Conditions

Cycling Stage 1: Hold 99°C for 300 sec

Cycling Stage 2: Two Temperatures x 40 Cycles of

99°C for 14 sec, Optics Off
64°C for 46.6 sec, Optics On

Channel Settings

475/520 channel	Unused	set Gain to 0	
530/565 channel	Unused	set Gain to 0	
585/630 channel	Pan-Aspergillus	set Gain to 60	set Threshold to 400
630/665 channel	Unused	set Gain to 0	
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.

Pan-Aspergillus

Open System Reagents for BD MAX™

REF 350-058-A-MAX

BioGX recommends setting a Ct max for the Pan-Aspergillus channel at 32.

Result Logic

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

Color Compensation

Color compensation settings are not required.

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

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.

Melt Settings

Melt settings are not needed for BioGX reagents.

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 other than the suggested sample processing outlined below. If different sample/specimen volumes are deemed appropriate for the existing workflow of the laboratory it will be necessary to modify Guardrail parameters accordingly.

Sputum and BAL specimens

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 specimens are outlined below. Note: Pretreatment reagents not included with BioGX 350-058-A-MAX.

Proteinase K Pretreatment: Pellet specimen (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. **SBT Loading:** 200 µL of the specimen is added to the SBT.

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 specimen, incubation time can range from 15 minutes to 120 minutes. **SBT Loading:** 200 µL of the specimen is added to the SBT.

Pan-Aspergillus

Open System Reagents for BD MAX™

REF 350-058-A-MAX

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 specimen, incubation time can range from 15 minutes to 30 minutes. **SBT Loading:** 200 µL of the specimen is added to the SBT.

Other Sample Types

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

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.

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™ 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 specimen to be tested.
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 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.
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.



Pan-Aspergillus

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REF 350-058-A-MAX

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.

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

Rev. #	Effective Date	Summary of Changes
02	21 MAY 2020	update BD MAX software version, add positive control part no., revise sample pretreatment, update instruction for using BioGX Sample-Ready™ Reagents on the BD MAX™, revise manufacturing designation, update UDP for compatibility with 4-Snap ExK-DNA-1
01	30 MAR 2018	Initial Release.