Candida auris
Open System Reagents for BD MAX™

REF 350-070-C-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 *Candida auris* 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, $MgCl_2$, 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-3 Open System extraction kits.

Basic suggested extraction processing parameters for: Urine (neat or boric acid preserved), Wound swab collected in Copan Universal Transport Media (UTM™) or Copan ESwab™, Pretreated sputum samples and Pretreated bronchoalveolar lavage (BAL) have been provided below.

Package Contents

Each 24-reaction package contains two pouches:

The first pouch contains 24 tubes of BioGX lyophilized reagents for *Candida auris* 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 RNA Beads (10^5 copies/bead)

-*Candida auris* - Part number 720-0173

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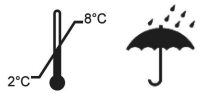
Storage Requirements and Recommendations



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 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-3 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) on to 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

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

BD MAX™ ExK™ DNA-3 for use with Urine (neat or boric acid preserved), Wound swab collected in Copan Universal Transport Media (UTM™) or Copan ESwab™, Pretreated sputum samples and Pretreated bronchoalveolar lavage (BAL).

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.

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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-3 - Urine (neat or boric acid preserved), Wound swab collected in Copan Universal Transport Media (UTM™) or Copan ESwab™, Pretreated sputum samples and Pretreated bronchoalveolar lavage (BAL) samples.

Sample Lysis Time	9 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™ extraction kit product insert.

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.

PCR Settings

475/520 channel	Unused	set Gain to 0	
530/565 channel	Unused	set Gain to 0	
585/630 channel	C. auris	set Gain to 60	set Threshold to 200
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.

Color Compensation

BioGX suggests the following initial color compensation settings:

1. Excitation Channel 585/630 with False Receiving Channel 630/665 – Set to "0"
2. Excitation Channel 680/715 with False Receiving Channel 630/665 – Set to "0"

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

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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.

Test Steps

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
58°C for 29.5 sec, Optics On
70°C for 14.5 sec, Optics Off

Result Logic

To simplify reports, result logic may be used 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 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.

Urine collection (neat urine or boric acid preserved urine)

Thoroughly vortex the sample prior to addition to the SBT. Add 500 µL of sample/specimen directly to the SBT. Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.

Wound swab [3 mL Copan Universal Transport Media (UTM™)]

Pipette 100 µL of specimen + 100 µL diluent (i.e. saline or phosphate buffered saline) into the Sample Buffer Tube (SBT), aseptically place the BDTM septum cap on each SBT. Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.

Wound swab [1 mL Copan Universal Transport Media (UTM™) or 1 mL Copan ESwab™]

Pipette 50 µL of specimen + 150 µL diluent (i.e. saline or phosphate buffered saline) into the Sample Buffer Tube (SBT), aseptically place the BDTM septum cap on each SBT . Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.

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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 350-068-C-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.

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.

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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.

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
01	12 MAY 2020	Initial Release.