



# BioCode<sup>®</sup>

## Fungal Panel

Package Insert



Applied BioCode

Barcoded Magnetic Beads | Flexible Syndromic Tests



## BioCode® Fungal Panel Package Insert

Part Number 64-F0001

For Research Use Only.  
Not for use in diagnostic procedures.

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## INTRODUCTION

### BioCode® Fungal Panel

The BioCode® Fungal Panel (FP) is a multiplex nucleic acid amplification test intended for use with the BioCode® MDx-3000 Instrument. The BioCode® FP is a qualitative assay capable of the simultaneous detection and identification of nucleic acids from the fungal pathogens (mold, dimorphic fungi, and yeast) listed in Table 1.

**Table 1. Organisms detected by the BioCode® Fungal Panel**

Mold	
▪ <i>Aspergillus fumigatus</i>	▪ <i>Cunninghamella bertholletiae</i>
▪ <i>Aspergillus terreus</i>	▪ <i>Lichtheimia corymbifera<sup>a</sup></i>
▪ <i>Aspergillus flavus</i>	▪ <i>Scedosporium apiospermum</i>
▪ <i>Aspergillus niger</i>	▪ <i>Lomentospora prolificans</i>
▪ <i>Mucor indicus</i>	▪ <i>Fusarium oxysporum</i>
▪ <i>Mucor circinelloides/racemosus</i>	▪ <i>Fusarium solani</i>
▪ <i>Rhizopus</i>	▪ <i>Syncephalastrum racemosum<sup>b</sup></i>
▪ <i>Rhizomucor</i>	▪ <i>Pneumocystis jirovecii</i>
Dimorphic Fungi	Yeast
▪ <i>Blastomyces dermatitidis</i>	▪ <i>Cryptococcus neoformans/gattii</i>
▪ <i>Coccidioides immitis/posadasii</i>	
▪ <i>Histoplasma capsulatum</i>	

<sup>a</sup>Assay may also detect some strains of other *Lichtheimia* species, such as *L. ramosa*.

<sup>b</sup>Assay may also detect some strains of other *Syncephalastrum* species, such as *S. monosporum*.

## SUMMARY AND EXPLANATION OF THE TEST

The BioCode® FP is a multiplex nucleic acid test intended for the qualitative detection of deoxyribonucleic acids (DNA) from 20 fungal pathogens. The BioCode® FP targets the highly conserved ITS2 region between the 5.8S and 28S rDNA for all fungi while *Pneumocystis jirovecii* is detected by its large mitochondrial subunit. Additionally, the assay targets a portion of the 18S fungal rDNA to detect the order Mucorales including *Rhizopus*, *Mucor*, and *Rhizomucor*. The BioCode® FP is designed for use with the BioCode® MDx-3000 automated system, and test results are available in about 4 hours post-DNA extraction. The test is for research use only and not for use in diagnostic procedures.

## PRINCIPLE OF PROCEDURE

The BioCode® MDx-3000 is an automated system that integrates PCR amplification, target capture, signal generation, and optical detection of nucleic acids for multiple fungal pathogens.

### Overview of a BioCode® MDx-3000 Run

- Multiplex PCR** – Purified nucleic acid solution is combined with a freshly prepared multiplex PCR reaction mix to enrich the target nucleic acids present in the sample. One of the primers in each primer pair is biotinylated at the 5'-end to generate labeled PCR product for subsequent detection.
- Dispensing BMB-Probe Mix** – Towards the end of PCR amplification, the instrument's robotic head dispenses BMB-Probe Mix into the designated reaction wells of the capture plate using disposable pipette

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tips.

3. **PCR Product Transfer** – After PCR amplification is completed, the robotic head pierces the foil seal with disposable pipette tips and transfers PCR products into the corresponding wells of the capture plate.
4. **Target Capture** – Amplified PCR products labeled with biotin are captured at a defined temperature by target-specific probes that are covalently coupled to designated Barcoded Magnetic Beads (BMBs). During this step, BMBs are kept in suspension by gentle agitation. Differentiation of captured targets is achieved by assigning a unique barcode pattern (BMB) for each pathogen and the internal control.
5. **Signal Generation** – After washing off unbound PCR products and unused primers, a streptavidin-phycoerythrin (SA-PE) conjugate is automatically added to the reaction by the robot. High affinity binding between biotin and streptavidin ensures that captured PCR products with the biotin moiety are labeled with phycoerythrin in close proximity to the BMBs.
6. **Optical Detection** – Optical detection is performed for each reaction well of the capture plate – an optically clear, 96-well flat-bottom microtiter plate. After washing off unbound SA-PE, excitation of the fluorophore at the designated wavelength emits fluorescence signal from BMBs tagged with SA-PE conjugates. Each reaction well is imaged at a specific emission wavelength for fluorescent signal and under bright field for identifying the barcode patterns (decoding).

The BioCode® MDx-3000 Software controls the operation of the instrument, collects and analyzes data, and automatically generates interpretation for test reports at the end of the run. Fluorescent signals from BMBs with the same barcode are sorted and calculated to generate median fluorescence intensity (MFI) for each analyte. For the BioCode® FP, the presence or absence of a pathogen can be determined relative to the MFI thresholds specified by the user. The software also analyzes the results of optional external and internal controls to validate the run and individual specimen results.

## MATERIALS REQUIRED

### Materials Provided with Each Kit

**Table 2.** Reagents provided in the BioCode® FP Assay Kit – enough for 96 tests

Component Name	Part No.	Contents	Storage
BioCode® Master Mix A	14-M0001	500 µL x 2	Store at -20°C before use. After thaw, store at 4°C for up to 30 days.
BioCode® Fungal Primer Mix	24-F0006	500 µL x 2	Store at -20°C before use. After thaw, store at 4°C for up to 30 days.
BioCode® DNA-IC	23-D0001	500 µL x 2	Store at -20°C before use. After thaw, store at 4°C for up to 30 days.
BioCode® Fungal BMB-Probe Mix	24-F0007	6000 µL x 1	Store at -20°C before use. After thaw, vortex for 30 sec, store at 4°C up to 90 days.

## Materials Required but Not Provided with Each Kit

**Table 3.** General Reagents required for the BioCode® MDx-3000

Component Name	Part No.	Contents	Storage
BioCode® SA-PE Mix	63-S0001	450 µL x 8	Single use; protect from light; store at 4°C. <b>Do Not Freeze</b>
BioCode® Buffer A	44-B0003	1 L x 1	Store at room temperature

**Table 4.** BioCode® MDx-3000 Consumables

Reagent	Source/Part No.	Quantity
Reagent Reservoirs	Applied BioCode 01-R0005 or INTEGRA 4332	50 each x 4
Waste Bin and Lid	Applied BioCode 01-W0104 and 01-W0105	25 each
20 µL pipette tips	Applied BioCode 01-P0006 or Beckman 717256	10 x 96 tips
250 µL pipette tips	Applied BioCode 01-P0007 or Beckman 717252	10 x 96 tips
Bio-Rad 96-well hard shell plate 0.1 mL	Applied BioCode 01-P0011 or Bio-Rad HSL9601	25 PCR plates
PCR Adhesive Foil	Applied BioCode 01-P0012 or Thermo Fisher Scientific AB-0626 or Eppendorf 0030127790	100 foils
Microtiter plate	Applied BioCode 01-P0009 or Greiner bio-one 655101	10 plates or 10 plates x 10 bags
Microtiter plate lid	Applied BioCode 01-P0010 or Nunc 5500	50 lids or 100 lids

### Equipment and Additional Consumables Required (but Not Provided)

- BioCode® MDx-3000
- Nucleic acid extraction method (e.g., MagNA Pure 96 [Roche], EMAG [bioMérieux])
- Vortex
- Centrifuge
- Pipettes – single, multi-channel and/or repeater with accuracy range between 1-10 µL, 10-200 µL, and 100-1000 µL
- Sterile, RNase/DNase-free disposable aerosol-barrier micro pipettor tips
- 1.5 mL polypropylene micro centrifuge tubes and racks (RNase/DNase free recommended)
- Cooler racks for 1.5 mL tubes and 0.1 mL 96 well plate
- Freezer (manual defrost) at -10 to -30°C
- Freezer (manual defrost) at -60 to -90°C
- Refrigerator at 2 to 8°C

## WARNINGS AND PRECAUTIONS

### General Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. The BioCode® FP Assay is to be used with the BioCode® MDx-3000.
3. Contamination may occur if carryover of samples is not adequately controlled during sample handling and processing or from the environment.

### Laboratory Precautions

1. The BioCode® FP Assay should be performed in clearly defined work areas moving in one direction from pre-amplification areas to the amplification/detection area to reduce potential for contamination.
  - a. Begin with reagent preparation before moving to amplification/detection.
  - b. Use dedicated equipment and supplies for each area (including personal protective equipment, such as lab coats and disposable gloves).
  - c. Clean work areas with 10% bleach or similar disinfectant followed by 70% ethanol before and after assay preparation.
2. A negative control is recommended for each run. If multiple lots are assayed at the same time, a negative control is recommended for each lot.
3. Do not use reagents past the expiration date. It is not recommended to mix reagents or interchange kit components from different kit lots. Kit configurations are identified on the kit outer box.
4. Assay setup should be performed at room temperature.

### Safety Precautions

1. Follow universal safety procedures.
2. Dispose of unused kit reagents according to local, state, and federal regulations.
3. Wear appropriate personal protective equipment including, but not limited to, lab coats, gloves, and protective eyewear. Change gloves often.
4. Safety Data Sheets for all reagents provided in the BioCode® FP can be accessed at <https://www.apbiocode.com/resources>.
5. Do not pipette by mouth.

## REAGENT STORAGE, HANDLING, AND STABILITY

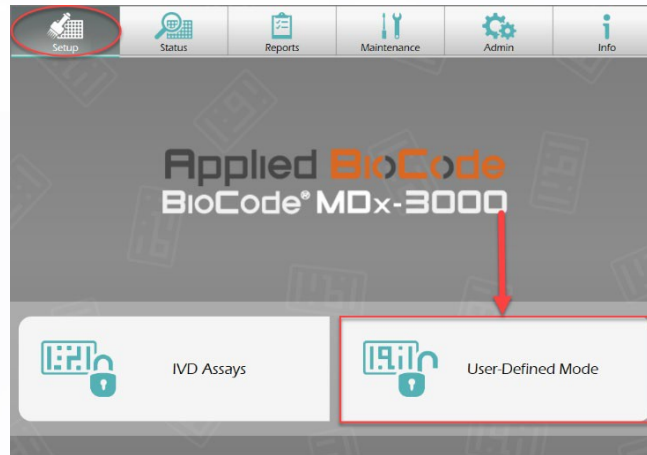
1. Store the kit components frozen (-30°C to -10°C) prior to use.
2. Once thawed, store Master Mix, Primer Mix, and DNA-IC refrigerated (2-8°C) for up to 30 days.
3. Once thawed, store BMB-Probe Mix refrigerated (2-8°C) for up to 90 days.
4. SA-PE mix is for single use only. Store refrigerated (2-8°C). Protect from light. **DO NOT FREEZE.**
5. Store Buffer A at room temperature (15-25°C).
6. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
7. Always check the expiration date and do not use reagents beyond the expiration date printed.
8. Remove BMB-Probe Mix from MDx-3000 once the run is complete and store refrigerated (2-8°C).

## MDx-3000 RUO PROTOCOL SETUP PROCEDURE

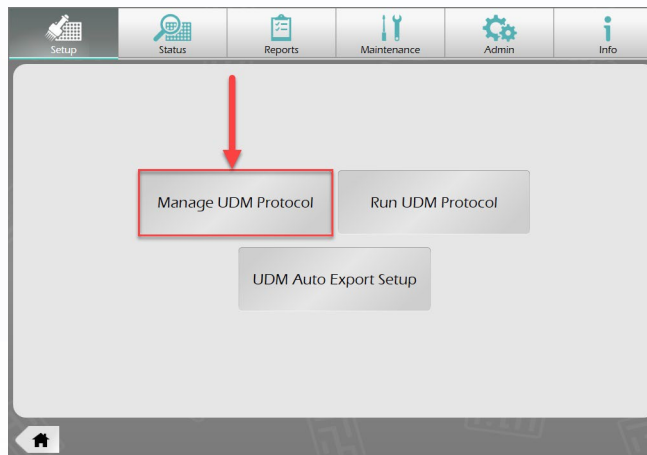
The BioCode® FP assay is designed to run with the User-Defined Mode of the MDx-3000 software. The user is responsible for creating and saving their own RUO protocol as well as determining their own assay threshold values and Minimum BMB Count.

Refer to the BioCode® MDx-3000 User Manual for more detailed instructions on how to set up an RUO run.

1. On the MDx-3000 software main screen, select User-Defined Mode.

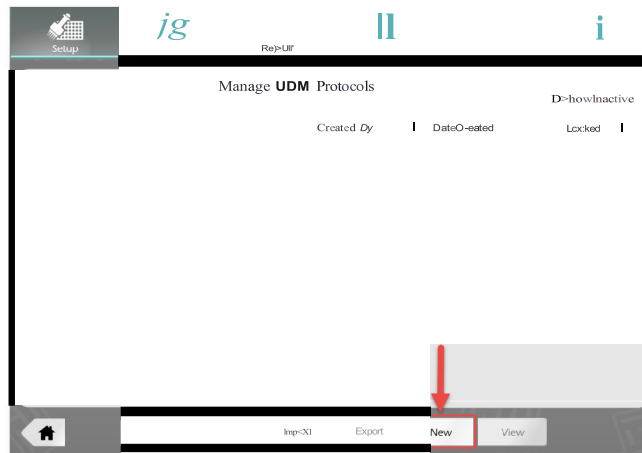


2. On the next screen, select Manage UDM Protocol.

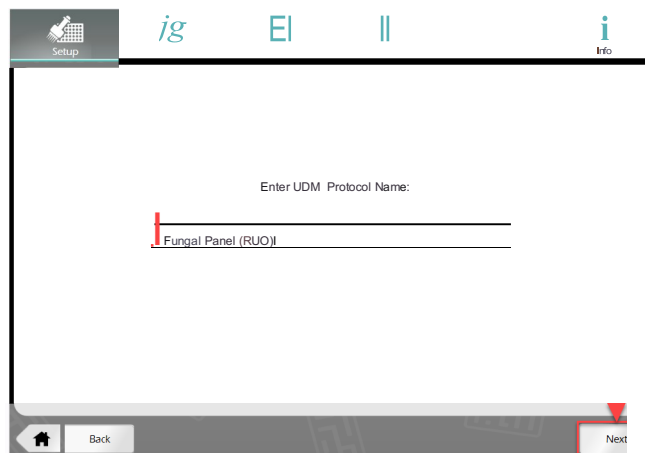




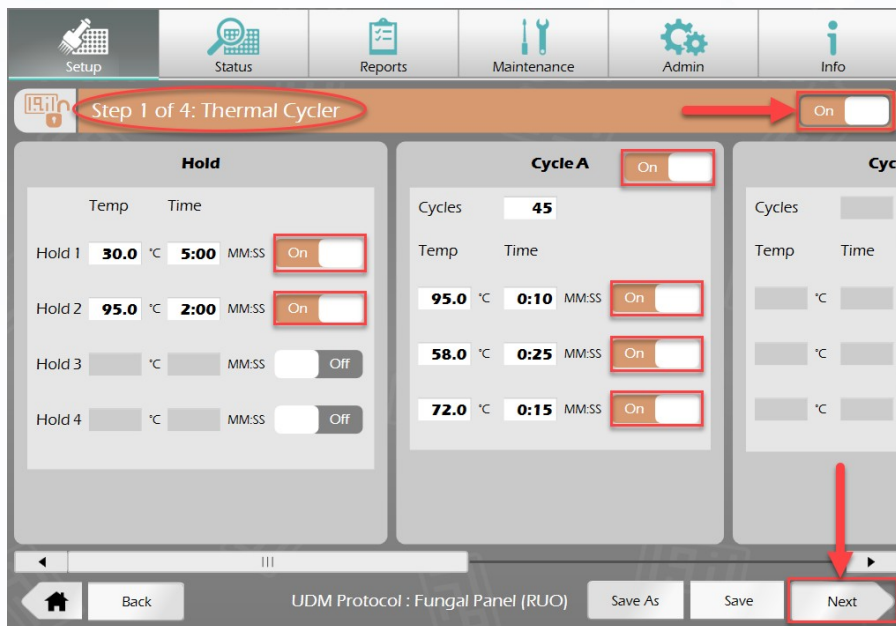
3. On the next screen, select New.



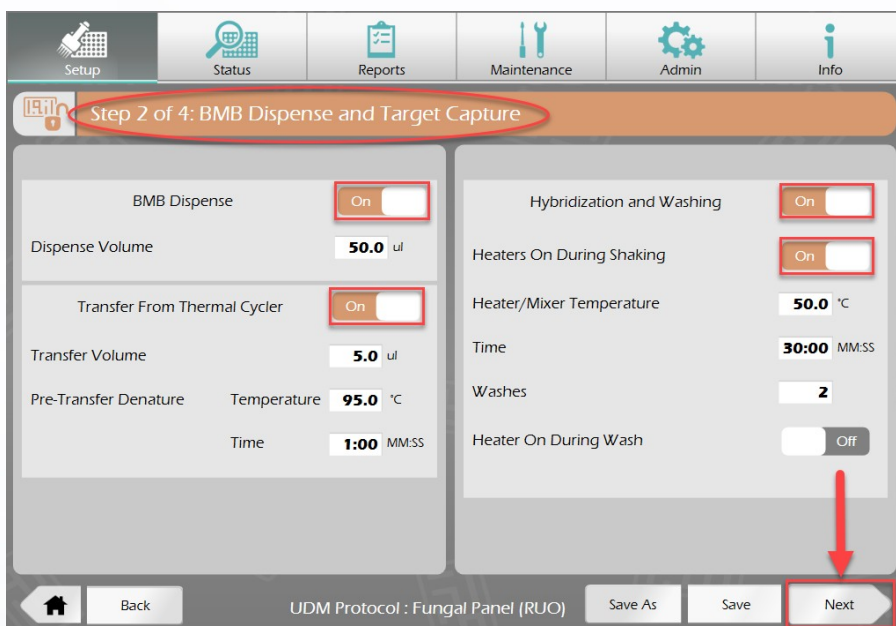
4. Enter a name for the RUO protocol and select Next.



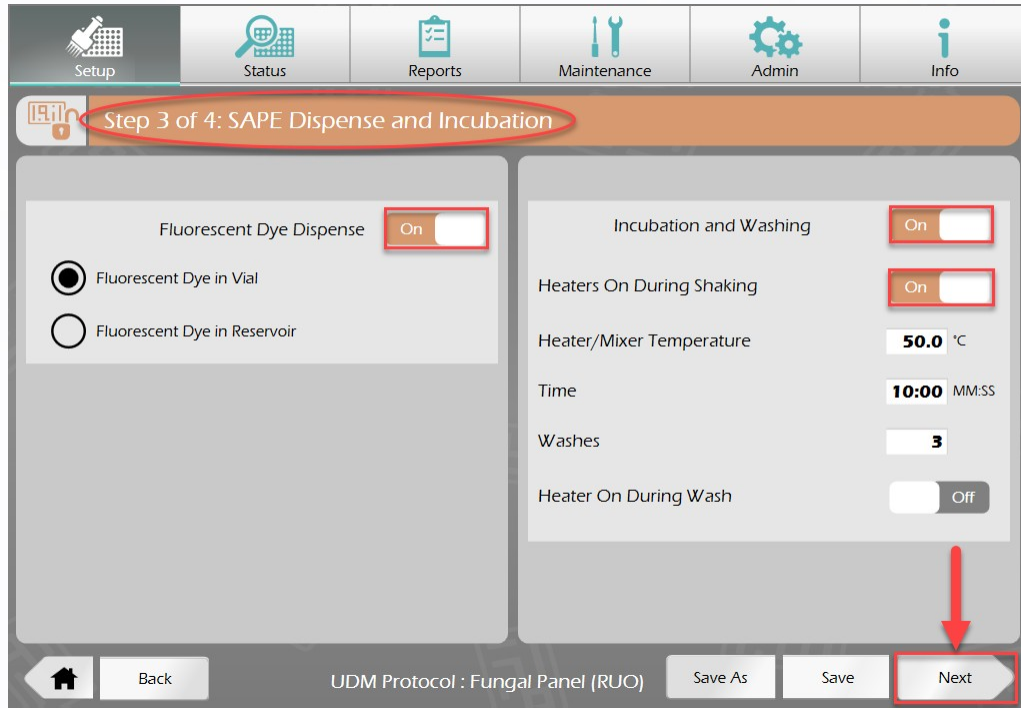
- For “Step 1 of 4: Thermal Cycler”, toggle the switch on the top right of the screen from Off to On and fill in the PCR cycling parameters as shown below. Select Next when finished.



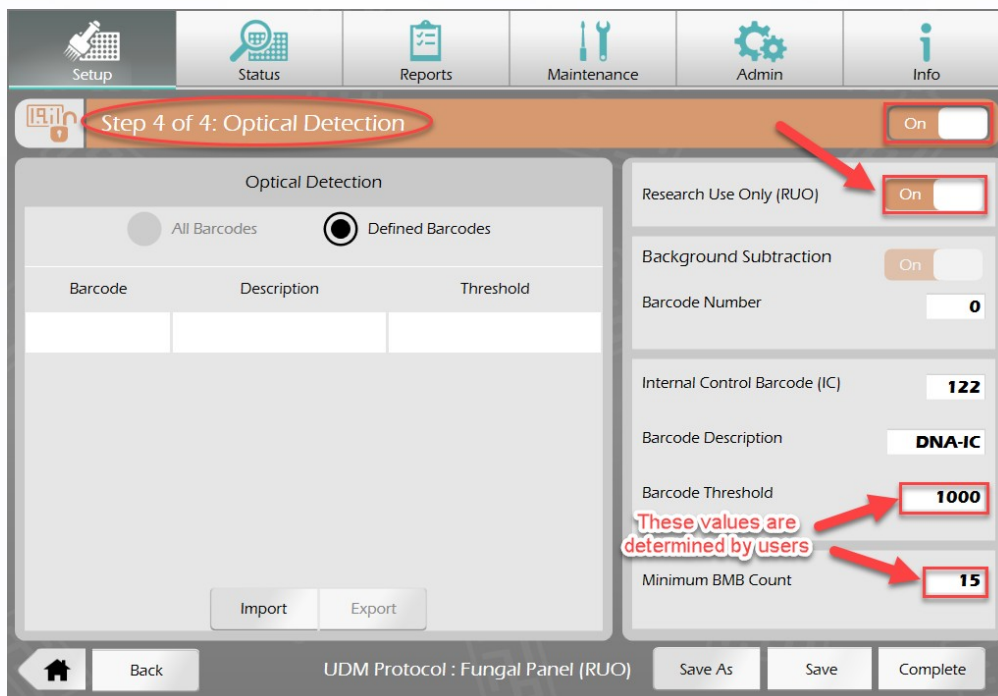
- For “Step 2 of 4: BMB Dispense and Target Capture”, fill in the BMB Dispense, Transfer From Thermal Cycler, and Hybridization and Washing parameters as shown below. Select Next when finished.



7. For “Step 3 of 4: SAPE Dispense and Incubation”, fill in the Fluorescent Dye Dispense and Incubation and Washing parameters as shown below. Select Next when finished.



- For “Step 4 of 4: Optical Detection”, toggle the switch on the top right of the screen from Off to On, then toggle the Research Use Only (RUO) switch from Off to On and fill in Background Subtraction Barcode Number, Internal Control Barcode (IC), and Barcode Description as shown below. Enter a Barcode Threshold value between 500 and 99999 for the Internal Control Barcode. For this Barcode Threshold, the screenshot provided below is only an example – the user is responsible for determining their own assay threshold value for the Internal Control. Enter a Minimum BMB Count value between 1 and 999. For Minimum BMB Count, the screenshot provided below is only an example – the user is responsible for determining their own Minimum BMB Count.



- On the left side of the screen, enter the Barcode and Description information from Table 5 below into the Optical Detection table.

**Table 5.** BioCode® FP Barcode numbers, descriptions, and detected targets.

Barcode <sup>a</sup>	Description	Target Detected
24	Lich spp	<i>Lichtheimia</i> spp.
25	Sync spp	<i>Syncephalastrum</i> spp.
26	M circ/race	<i>Mucor circinelloides/racemosus</i>
27	Mucorales	Mucorales
32	A fum	<i>Aspergillus fumigatus</i>
33	A terr	<i>Aspergillus terreus</i>
34	A flav	<i>Aspergillus flavus</i>
35	A nig	<i>Aspergillus niger</i>
36	C bert	<i>Cunninghamella bertholletiae</i>
37	M ind	<i>Mucor indicus</i>
38	Rhizomuc spp	Rhizomucor spp.
39	Rhizopus spp	Rhizopus spp.
42	P jiro	<i>Pneumocystis jirovecii</i>
43	F oxy	<i>Fusarium oxysporum</i>
44	F sol	<i>Fusarium solani</i>

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47	S apio	<i>Scedosporium apiospermum</i>
48	L pro	<i>Lomentospora prolificans</i>
59	Crypto spp	<i>Cryptococcus spp.</i>
60	B derm	<i>Blastomyces dermatitidis</i>
61	C imm/posa	<i>Coccidioides immitis/posadasii</i>
62	H cap	<i>Histoplasma capsulatum</i>

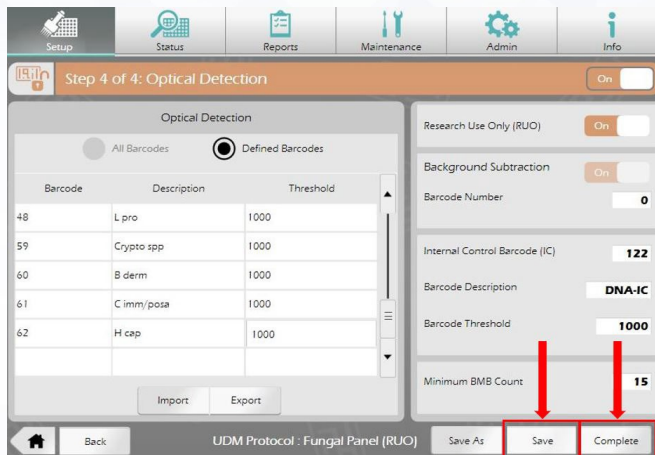
<sup>a</sup>Barcode/target thresholds to be determined by user

10. Enter threshold values for each barcode into the Optical Detection table. The screenshots provided below are only an example. The user is responsible for determining their own assay threshold values for each barcode in the panel.

The screenshots show the 'Optical Detection' interface with the following data:

Barcode	Description	Threshold
24	Lich spp	1000
25	Sync spp	1000
26	M circ/race	1000
27	Mucorales	1000
32	A fum	1000
33	A terr	1000
34	A flav	1000
35	A nig	1000
36	C bert	750
37	M ind	1000
38	Rhizomuc spp	750
39	Rhizopus spp	1100
42	P jiro	1000
43	F oxy	1000
44	F sol	1000
47	S apio	1000
48	L pro	1000
59	Crypto spp	1000
60	B derm	1000
61	C imm/posa	1000
62	H cap	1000

11. When finished with Step 4 of 4, click Save. Then, click Complete.



## ASSAY PROCEDURE

The starting material for the BioCode® FP assay is purified nucleic acid. The user should determine the method of nucleic acid purification used and should ensure that method is compatible with PCR. The BioCode® DNA-IC provided in the kit should be added to each sample prior to extraction and is used as an extraction, amplification, and detection control for the BioCode® FP.

A negative control is recommended for each run. If multiple BioCode® FP kit lots are assayed at the same time, a negative control is recommended for each kit lot. A negative control may consist of extracted negative matrix or a well characterized negative sample spiked with BioCode® DNA-IC.

Gloves and other Personal Protective Equipment (PPE) should be used when handling reagents. Once PCR reagents are prepared and sample is added to the PCR plate, the plate should be promptly transferred to the instrument to start the run. After the run is complete, the PCR plate and capture plate should be sealed and discarded.

Refer to the BioCode® MDx-3000 User Manual for more detail and pictorial representations of the BioCode® MDx-3000 User-Defined Mode run set up instructions, as well as instructions for viewing the RUO results reports.

### **Nucleic Acid Purification (for Example Purposes Only)**

**Note: The method used for nucleic acid purification is determined by the user. This section is provided only as an example.**

1. Prepare samples of interest by combining 200 µL sample, 400 µL 1x PBS buffer, and 10 µL DNA-IC into a bead beating tube.
2. Vortex the tube with beads on high for 10 minutes. Briefly spin down the tube in a centrifuge.
3. Extract nucleic acids with a method selected by the user.

### **BioCode® FP Assay Setup**

**Note: Prepare the PCR Plate in a dedicated reaction mix preparation area.**

1. Thaw Primer Mix, Master Mix A, and BMB-Probe Mix at room temperature. For the Primer Mix and

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Master Mix A, perform a quick vortex (2-3 seconds) and centrifuge to collect reagents at the bottom of the tubes.

2. Prepare the reaction mix in a polypropylene microcentrifuge tube as described below:

**Table 6.** Reaction Mix Formulation

Component	Reaction Mix Volume (µL) per reaction	Reaction Mix Volume (µL) per 10 reactions
BioCode® Master Mix A	10.0 µL	100.0 µL
BioCode® Fungal Panel Primer Mix	10.0 µL	100.0 µL
<b>Reaction Mix Volume (µL)</b>	<b>20 µL</b>	<b>200 µL</b>

3. Mix reaction mix by briefly pulse vortexing or pipetting up and down 8 to 10 times. Centrifuge to collect contents at the bottom of the tube. If proceeding immediately to PCR plate setup, reaction mix may be kept at room temperature. If not proceeding immediately to PCR plate setup, store reaction mix at 2-8°C or in a cooling block for up to 1 hour.
4. Pipette 20 µL of reaction mix into the appropriate wells of a 96-well plate.
5. Pipette 5 µL of each extracted sample into the wells.
6. If using extracted negative control(s), pipette 5 µL of the negative control(s) into the negative control well(s).
7. Seal plate with a pierceable foil. Store at 2-8°C or on a cooling block until ready to load onto the BioCode® MDx-3000 (not to exceed one hour from the time the reaction mix is prepared).
8. Briefly centrifuge plate to collect samples at the bottom of the plate.
9. Load plate onto the BioCode® MDx-3000.
10. Vortex BMB-Probe Mix for 30 seconds at high speed and load the vial onto the BioCode® MDx-3000. **(Note: Precipitates may appear at cold temperatures. If precipitates are present, allow the BMB-Probe Mix to warm to room temperature and vortex additional 30 seconds.)** Based on the number of wells required in the plate setup, the BioCode® MDx-3000 software calculates the bulk BMB-Probe Mix volume required and displays the volume on the screen. The BioCode® MDx-3000 automatically dispenses 50 µL of BMB-Probe Mix into each well of the optical detection plate, based on the plate setup.
11. Load reagents and consumables as prompted by graphic user interface. Refer to the BioCode® MDx-3000 User Manual for more details on RUO run setup. Start the Run.

## INTERPRETATION OF RESULTS

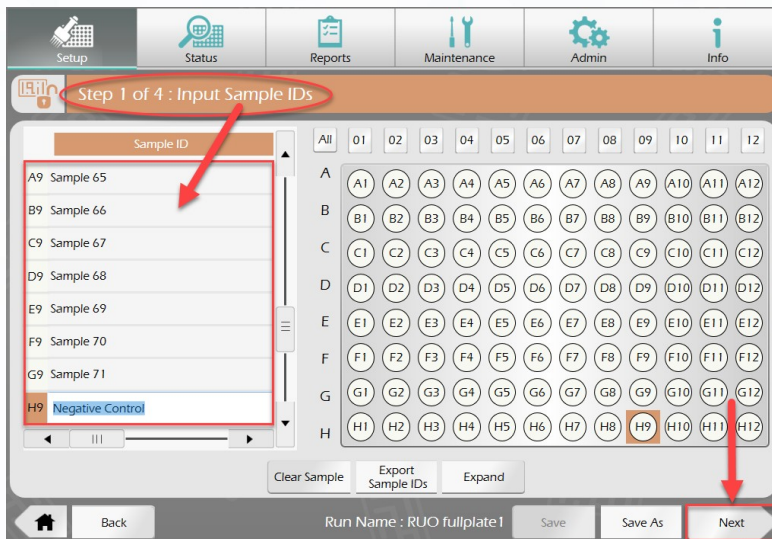
The BioCode® MDx-3000 software will analyze data based on plate validity, sample validity, and Median Fluorescent Intensity (MFI) compared to the MFI thresholds specified by the user during the MDx-3000 RUO protocol setup.

### External Control

An external negative control is recommended and can be negative sample matrix or well characterized negative samples spiked with the provided DNA-IC. If the user decides to include a

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negative control in a BioCode® FP run, it should go through all processing steps (extraction, amplification, and detection). During run setup, an external negative control can be defined by naming any sample “Negative Control” (case insensitive) in the Sample ID column on the left side of the “Input Sample IDs” screen, as exemplified in the screenshot below.



## **Internal Control**

It is recommended that the user add the DNA Internal Control (DNA-IC) supplied in the kit to each sample and negative control during extraction. The internal control monitors the efficiency of the extraction, amplification, and detection stages of the assay. Lack of DNA-IC signal may indicate sample-associated inhibition or reagent/instrument issues. Samples suspected of being inhibitory can be repeated from extraction. If reagent or instrument issues are suspected, samples may be repeated from stored nucleic acid extracts.

## **Target Pathogen Interpretation**

Fluorescent signals from BMBs with the same barcode are sorted and the median fluorescence intensity (MFI) is calculated for each analyte. The BioCode® MDx software interprets the results based on the MFI threshold values set by the user during the initial MDx-3000 RUO protocol setup.

If the user decides to include controls in a run, such as DNA-IC in extracted samples and one or more external controls, it is recommended that the user examine the results of the control(s) prior to assessing the results for all other samples.

## **BioCode® Fungal Panel Reports**

The analyzed BioCode® MDx-3000 results are displayed in four report formats: MFI Report, BMB Count Report, RUO Report, and Sample Report. All reports can be reviewed electronically and exported as a PDF or CSV file. The MFI Report and BMB Count Report display raw results for specimens and controls, while the RUO Report and Sample Reports contain interpreted results. Refer to the BioCode® MDx-3000 User Manual for more details and example screenshots of BioCode® MDx-3000 reports.

The MFI Report displays the raw MFI values calculated for each well. The user may compare the raw MFIs in the MFI Report to the barcode-specific MFI thresholds selected during the initial MDx-3000 RUO



protocol setup.

The BMB Count Report displays the BMB counts for each barcode in each well. The user may compare the raw counts in the BMB Count Report to the Minimum Bead Count specified during the initial MDx-3000 RUO protocol setup.


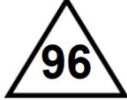







The RUO Report displays interpreted results in a tabular format for each well. Possible results by target are Detected, Not detected, or Invalid.

The Sample Report displays results for a single well. In addition to results for each target, the Sample Reports include a results summary section which allows positive results to be reviewed at a glance. The Sample Report results summary indicates well validity based on BMB counts, background MFI, and external and internal controls. Sample Reports also include any sample-specific comments entered during run setup.

All report headers provide traceability information for the unique Run Name, RUO Protocol used, Run Start and Run Complete times, User, Run Status, SA-PE Mix lot (if entered by user during run setup), Wash buffer lot (if entered by user during run setup), Software Version/Mode, Instrument, and External Controls. The Run Status section will specify if the run is Valid, Invalid, Complete, or Incomplete based on the negative control results (if using one or more negative controls) for the specific run/kit lot. The External Controls section indicates the results for the negative controls (Valid, Invalid, or N/A). It is recommended to review the Run Status and External Controls sections prior to reviewing target results.

## TABLE OF SYMBOLS

The following symbols are used on the BioCode® FP Assay components and/or in this package insert.

	Batch code		Contains 96 tests		Temperature limitations
	Part Number		Warning		Consult instructions for use
	Use by YYYY-MM-DD		Registered trademark		Manufacturer

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