Dynamiker Biotechnology (Tianjin) Co., Ltd.

MycoMDx *Candida* PCR Assay

Catalogue No.: DNK-1417-1

User Manual / 50 tests

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1. INTENDED USE

The MycoMDx *Candida* PCR Assay is a multiplex polymerase chain reaction (PCR) *in vitro diagnostic* (IVD) test for the detection of *Candida* DNA extracted from EDTA whole blood, EDTA plasma, serum obtained from human patients considered at risk of invasive fungal disease (IFD). This kit established a method that can simultaneously detect five common pathogenic *Candida* clinically, including *Candida albicans, Candida tropicalis, Candida krusei, Candida glabrata, Candida parapsilosis*. This method has high sensitivity and specificity, which is of great significance for the early diagnosis and treatment of invasive *Candida* infections.

2. PRODUCT INTRODUCTION

2.1 Invasive Fungal Disease

Invasive fungal disease (IFD) are fungal infectious disease caused by pathogenic fungi invading subcutaneous tissues, mucous membranes, muscles and internal organs, etc. Candida spp. have 81 species, 11 of which are pathogenic to humans. Candida is the most common fungus among opportunistic or conditional pathogenic fungi, and fungal infections caused by Candida are the leading among invasive fungal diseases (IFD). Healthy human have strong immunity to it. In recent years, due to the extensive development of hematopoietic stem cell transplantation and solid organ transplantation, the use of high-intensity immunosuppressive agents and a large number of chemotherapy drugs, in vivo interventional examination and treatment of various catheters, as well as the implantation and retention of great venous catheters and urinary catheters, the incidence of IFD increased significantly in clinically.

The diagnosis of invasive fungal diseases is extremely challenging, ranging from the initial atypical clinical manifestations to diagnostic difficulties, leading to the considerable variation in case definitions. Although the biomarkers of fungi are widely increasing, but they are still only used for clinical prevention and empirical treatment. Diagnosis of invasive fungal disease by PCR is superior to existing methods in terms of speed, sensitivity and specificity. At present, diagnosis is mainly used in combination with microscopy and serology (testing of galactomannan (GM) and beta-d glucan).

The MycoMDx *Candida* PCR Assay is a multiplex real-time PCR in vitro test for qualitative detection of *Candida* DNA extracted from EDTA whole blood, EDTA plasma，serum. The test can help early diagnosis by provide rapid and reliable testing, while quality control ensures that

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users can have full confidence in the quality and reproducibility of the test.

2.2 Kit Components

The number of reagents can be used for 50 tests.

Reagent	Volume (μL)	Cap color	Cap number
PCR Master Mix	300	Orange	1
PCR PP	800	brown	2
Internal Control	300	pink	3
PCR Water	1200	white	4
Positive Control	600	red	5
Negative Control	600	blue	6

The MycoMDx Candida PCR Color Compensation Kit is provided separately (For use with LightCycler 480 II instruments).

3. STORAGE AND EXPIRATION DATE

3.1 Inspection on Arrival

Check the MycoMDx *Candida* PCR Assay on arrival. If the packaging is damaged the kit must not be used. In addition, the reagents should be transported at 2-8 °C or lower temperature.

3.2 Storage Space and Temperature

The kit should be stored in an amplicon free laboratory. Positive control (PC), Negative control (NC) and Internal control (IC) in the kit should be stored in the area where the DNA template/sample template is stored. Should be kept in-25 °C to -15 °C, valid for one year.

3.3 Stability

The MycoMDx *Candida* PCR Assay showed that under the condition of more than 10 freeze-thaw cycles, its performance is not affected. All reagents contained within the kit have been marked on the kit label and must be used within this effective period.

4. MATERIALS REQUIRED BUT NOT PROVIDED

4.1 General Equipment

Real-time quantitative PCR instrument (over four channels)

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Vortex mixer

Mini centrifuge

Microplate processing centrifuge

Refrigerator (2-8 °C) and freezer (-25 °C to -15 °C)

Micropipettes for volumes of 1 to 1000 µL

4.2 Reagents

Recommended nucleic acid extraction reagent

4.3 Consumables

DNase/RNase free plastics were used in the preparation of PCR

DNase/RNase free 1 to 1000 µL pipette tips

Disposable gloves, powderless

Disposable face mask

5. SAMPLE COLLECTION AND DNA EXTRACTION

The quality of extracted DNA is essentially related to the sensitivity of real-time PCR detection, so samples must be collected and stored according to the following guidelines.

Precise experimental practice recommends including at least one positive and negative control extraction in each analysis.

Extracted samples should be stored at -80 °C for long-term storage, in -25 °C to -15 °C for temporary storage.

Roche's High Pure PCR Template Preparation Kit. bioMeriere's NucliSENS easyMAG Extraction Reagents. Qiagen's Fully Automatic Assay Setup System are recommended for DNA extraction.

6. TEST PROCEDURE

6.1 PCR Instrument Setting

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It is recommended that a clean laboratory to set up all PCR reactions. To avoid any risk of contamination, users should always follow standard laboratory practices and isolate operational processes properly.

Before starting the PCR setting, the user should prepare the required consumables such as 96-well plates, the sample will be precisely assigned to the appropriate wells in advance. The information should be recorded at any time.

6.1.1 PCR Reaction Procedure 1

The process of extracting nucleic acid put in Internal Control(IC), setting procedure as follows:

Remove the MycoMDx *Candida* PCR Assay from the refrigerator and allow the reagents to thaw.

Prepare PCR reaction Mix by referring to the reagents and volumes shown in table 1. The volume of each reaction well should be multiplied by the number of reaction wells. At the same time taking into account the number of samples in the analysis. The prepared PCR reaction Mix should be fully mixed and centrifuged for 10 seconds.

Table 1 Preparation of PCR reaction volume without adding clinical sample

Reagent component	PCR addition volume (μL)
PCR Master Mix	5
PCR PP	15
PCR Water	0
Internal Control	0
Total volume（per well）	20

For clinical samples, in each reaction well add 20 μL PCR reaction Mix firstly (see table 1) and in each reaction well add 5μL extracted Sample (see table 2).

Table 2 Final PCR reaction volume after adding clinical samples

Reagent component	PCR addition volume (μL)
Total volume（per well）	20
Sample	5
Final volume（per well）	25

For the Positive Control and Negative Control, prepare PCR reaction Mix according to the

reagent and volume shown in table 3 and the prepared PCR reaction Mix should be fully mixed and centrifuged for 10 seconds. In each reaction wells add 20 µL PCR reaction Mix respectively. (see table 3).

Table 3 Preparation of PCR reaction volume before adding Positive Control/Negative Control

Reagent component	PCR addition volume (µL)
PCR Master Mix	5
PCR PP	15
PCR Water	0
Internal Control	1
Total volume （per well）	21

For each Positive Control, add 4 µL Positive Control to the PCR reaction Mix. Similarly, for each Negative Control, add 4 µL Negative Control to the PCR reaction Mix. Include at least one Positive Control and one Negative Control.

Table 4 Final PCR reaction volume after adding positive control/negative control

Reagent component	PCR addition volume (µL)
Total volume （per well）	21
Positive/ Negative Control	4
Final volume （per well）	25

Then the entire 96-well plate is placed in the centrifuge to ensure that the sample is centrifuged rapidly. The 96-well plate is placed into a PCR instrument to initiate the PCR process.

6.1.2 PCR Reaction Procedure 2

The process of extracting Nucleic acid does not put in Internal Control (IC), setting procedure as follows:

Remove the MycoMDx *Candida* PCR Assay from the refrigerator and allow the reagents to thaw.

Prepare PCR reaction Mix by referring to the reagents and volumes shown in table 5. The volume of each reaction well should be multiplied by the number of reaction wells. At the same time taking into account the number of samples in the analysis. The prepared PCR reaction Mix should be fully mixed and centrifuged for 10 seconds.

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Table 5 Preparation of PCR reaction volume without adding clinical sample

Reagent component	PCR addition volume (μL)
PCR Master Mix	5
PCR PP	15
PCR Water	0
Internal Control	1
Total volume （per well）	21

At first, in each reaction well add 20 μL PCR reaction Mix (see table 5). For clinical sample, in each reaction well add 5 μL extracted Sample, add 5 μL Negative Control or Positive Control to the PCR reaction respectively for the Positive Control and Negative Control (see table 6).

Table 6 Final PCR reaction volume after adding Positive Control/Negative Control

Reagent component	PCR addition volume (μL)
Total volume （per well）	21
Positive/ Negative Control	4
Final volume （per well）	25

Then the entire 96-well plate is placed in the centrifuge to ensure that the sample is centrifuged rapidly. The 96-well plate is placed into a PCR instrument to initiate the PCR process.

7. PROCEDURE SETTING

7.1 LightCycler 480 II PCR protocol

Please refer to the instruction on how to operate LightCycler 480 II Real-Time PCR instrument and data analysis.

A detailed list about different detection channels corresponding to the detection targets on Table 7. Set these dyes to report dyes.

Table7 Detector channels used to detect the corresponding of *Candida* targets using the LightCycler 480 II

	Red	Orange	Green	Yellow
Dye Channel	FAM	HEX/VIC	ROX	Cy5

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Targets	Internal Control	*Candida* spp./*Candida albicans*	*Candida glabrata/Candida parapsilosis*	*Candida krusei/Candida tropicalis*

Set the detection format and use LightCycler 480 II to set the following settings:

Reaction volume: 25 μL. The test procedure is shown in table 8. Data of 45 cycles should be collected.

Table 8 PCR parameters for the MycoMDx *Candida* PCR Assay

Step	Temperature (℃)	Data collection	Time	Number of cycles	Analysis Mode
Pre-denaturation	95	None	2 min	1	None
Extension	95	None	10 sec	40	Quantification
	60	Single	40 sec		
Extension	95	None	5 sec	5	None
	65	None	20 sec		
Melting Curves	95	None	60 sec	1	None
	70	None	120 sec		Single
	50	None	120 sec		
	85	Continuous	0.04℃/s		
Cooling	37	None	10 sec	1	None

7.2 ABI7500 PCR protocol.

Please refer to the instructions on how to operate ABI7500 Real-Time PCR instrument and data analysis.

A detailed list about different detection channels corresponding to the detection targets on Table 9. Set these dyes to report dyes.

Table 9 Detector channels used to detect the corresponding of *Candida* targets using the ABI 7500

	Red	Orange	Green	Yellow
Dye Channel	FAM	HEX/VIC	ROX	Cy5
Targets	Internal Control	*Candida* spp./*Candida albicans*	*Candida glabrata/Candida parapsilosis*	*Candida krusei/Candida tropicalis*

Set the detection format and use ABI7500 to set the following Settings:

Reaction volume: 25 μL. The test procedure is shown in table 10. Data of 45 cycles should be collected.

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Table 10 PCR parameters for the MycoMDx *Candida* PCR Assay

Step	Temperature (℃)	Data collection	Time	Number of cycles	Analysis Mode
Pre-denaturation	95	None	2 min	1	None
Extension	95	None	10 sec	40	Quantification
	60	Single	40 sec		
Extension	95	None	5 sec	5	None
	65	None	20 sec		
Melting Curves	95	None	60 sec	1	None
	70	None	120 sec		Single
	50	None	120 sec		
	85	Continuous	1%		

Finally, analyzing data in the option box, cancel the automatic threshold and automatic baseline of each target and conduct manual setting. Set the threshold of each target channel in the Analysis Settings window refer to table 11 for details.

Table 11 ABI 7500 Threshold values for the MycoMDx *Candida* PCR Assay

Dye Channel	FAM	HEX/VIC	ROX	Cy5
Targets	Internal Control	*Candida* spp./*Candida albicans*	*Candida glabrata/Candida parapsilosis*	*Candida krusei/Candida tropicalis*
Threshold	15000	15000	50000	10000

8. INTERPRETATION OF RESULTS

8.1 Internal Control

Internal Control and *Candida* species detection are completely different channels and internal control should be stable at a specific value. If the internal control fails but the samples report as positive for one or more *Candida*, the positive result is also valid.

If all samples report as negative and internal controls are negative, the same extract should be used for repeated testing. If the internal control is still negative after repeated test, the sample should be tested again from the extraction step.

8.2 Positive and Negative Controls

At least one positive and negative control provided in the kit should be included in each analysis. Each negative or positive control should be prepared and tested in the same way as the patient samples.

Negative control produce positive test results indicating samples contamination problem. A new

"negative control material" should be repeated to ensure proper decontamination of the operating area and equipment.

Positive control that produce negative results indicate problems with the reagent or a wrong sample addition. Ensure that all reagents are stored correctly and tested within their expiry dates.

Samples sent for testing via the MycoMDx *Candida* PCR Assay may have significantly varied loads of material present and much of this may be very close to the limit of detection of the biomarker, therefore any Ct value less than 38 for the corresponding *Candida* species may indicate a positive sample. Table 12 summarizes the results possible with the MycoMDx *Candida* PCR Assay.

Table 12 Analysis of detection results for the MycoMDx *Candida* PCR Assay

Channel	Target MycoMDx *Candida*	C_t value	Tm value	Result interpretation
Red	Internal Control	10≤Ct<38	N/A	Pass
		N/A or <10 or≥38	N/A	Fail
Orange	*Candida* spp./*Candida albicans*	≤40	60≤Tm≤67	*Candida* spp.
		≤40	59≤Tm≤62	*Candida albicans*
		N/A	-	Negative sample
Green	*Candida glabrata/Candida parapsilosis*	≤40	73≤Tm2≤77	*Candida glabrata*
		≤40	64≤Tm≤67	*Candida parapsilosis*
		N/A	-	Negative sample
Yellow	*Candida krusei/Candida tropicalis*	≤40	64≤Tm≤67	*Candida krusei*
		≤40	74≤Tm≤77	*Candida tropicalis*
		N/A	-	Negative sample

9. TROUBLESHOOTING

1. The Positive Control is not within the normal range

Reasons：The kit did not store in storage section of this Instruction for Use (IFU), or the kit has expired. One of the components was not added or incorrect PCR profile/programming.

Advices：Please check correct kit storage conditions has been followed. Check the expiry date of the kit box. Ensure that all components have been added and check your work steps procedure and check calibration of real-time PCR machine.

2. The Negative Control has generated a positive signal in one or more channels.

Reasons：Contamination occurred during the adding process.

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Advices：Taking extra care when adding the templates, in particular the positive control. Make sure that the work area and instruments are properly decontaminated before and after use.

3. IC remains negative in *Candida* negative samples

Reasons：Nucleic acid degradation. DNA extraction procedure is used which is not validated and results in less efficient IC extraction yield.

Advices：Repeat the extraction according to the protocol. Using the particular DNA extraction platform.

4. Very weak fluorescence signals

Reasons：Incorrect instrument settings. Incorrect real-time PCR mix

Advices：Check channel settings. Check if the PCR mixtures are prepared according to the protocol. Check expiry date and storage conditions.

10. PERFORMANCE CHARACTERISTICS

The performance characteristics identified throughout verification and validation of the MycoMDx *Candida* PCR Assay are highlighted in Table 13.

Table 13 Clinical performance characteristics of the MycoMDx *Candida* PCR Assay

Sample type	Characteristics (%)	Result
Simulated serum samples	Sensitivity (%)	95.12
	Specificity (%)	95.75
Simulated Plasma samples	Sensitivity (%)	96.32
	Specificity (%)	96.52
Simulated Whole Blood Samples	Sensitivity (%)	100
	Specificity (%)	100

The low limit detection of this product is 2×10^3 copies/mL.

The coefficient of variation (CV) values of intra batch error of this kit are less than 5%.

It has been proved that this product will not cross-react with other types of pathogens commonly in clinical practice, such as *Aspergillus fumigatus, Aspergillus flavus,Aspergillus terreus, Aspergillus niger, Bordetella pertussis, Escherichia coli, Moraxella catarrhalis*,

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Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae etc., but there might be a cross-reaction between them *Penicillium marneffei, Penicillium rosea* and *Penicillium citrinum.*

11. RISK AND SAFETY INFORMATION

The kit contains no harmful substances. The composition of all reagents in the kit poses no specific risk to the user or his property. Other chemicals and materials may be required for the procedures used in this instruction. Read carefully any warnings, instructions or material safety data sheets provided by the supplier and comply with general safety regulations when handling chemicals, biological hazards or other materials.

11.1 General Precautions

1. The product is ONLY for IVD use.

2. Do not use the kit if the label seal is broken upon receipt of the product. Before beginning the test, the packaging and sealing of the product as well as its shelf life must be checked. The product cannot be used after the specified period of validity.

3. Reagents from different test tubes or kits should not be mixed at will even if they come from the same batch. Also do not replace reagents from different manufacturers.

4. Long-term storage of low-concentration DNA is unstable, so the storage time of samples should be shortened as much as possible.

5. The samples tested by the kit are whole blood, plasma and serum.

6. The testing procedure should be carried out in accordance with the requirements of this instruction.

7. Ensure all required consumables are DNase/RNase free.

11.2 Biological risks

The MycoMDx *Candida* PCR Assay involves potentially dangerous and transmissible biomaterials. People who using the kit must read and follow all necessary health and safety precautions.

It is important to wear appropriate personal protective equipment during operation. At a minimum, laboratory clothing, protective gloves, respirator and safety glasses are required.

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12. DISPOSAL

Proper handling this production will help to conserve natural resources. Be sure to recycle it in a way that prevents potential negative impacts on the environment and human health.

General disposal

Please dispose of unused reagents, wastes and transport materials in accordance with national and local regulations.

Packaging disposal

Please dispose of all packaging according to local recycling regulations.

13. MANUFACTURER

Company: Dynamiker Biotechnology (Tianjin) Co., Ltd

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Website: www.dynamiker.com

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Company Name: Lotus NL B.V.

Address: Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.

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[**SYMBOLS USED**]

The following symbols may appear on the label of the MycoMDx *Candida* PCR Assay or in the instructions for use.

Symbol	Description
	Use By
	Batch Code
	Manufacturer
	Keep Away from Sunlight
	Temperature Limitation
	*In vitro diagnostic* Medical Device
	Product reference number
	Authorized Representative in the European Community
	CE Mark

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