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Candida albicans Real Time PCR Kit User Manual

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For use with ABI Prism®7000/7300/7500/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/Chromo4; LightCycler®480 Instrument

LifeTechnologies(India)PvtLtd.
306,AggarwalCityMall,OppositeM2KPitampuraNewDelhi-110034(INDIA)Ph:+91-11-42208000, 42208111,42208222,Mobile:+91-9810521400,Fax:+91-11-42208444
Email:customerservice@lifetechindia.com Website:www.lifetechindia.com

1. Intended Use

Candida albicans Real Time PCR Kit is used for the detection of Candida albicans in genital swabs，sputum, nasopharyngeal swabs, urine and any other kind of sample from which can be extracted DNA by using real time PCR systems.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans, and candidal onychomycosis, an infection of the nail plate.

C. albicans is commensal and a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract. C. albicans lives in 80% of the human population without causing harmful effects, although overgrowth of the fungus results in candidiasis (candidosis). Candidiasis is often observed in immunocompromised individuals such as HIV-infected patients.

The Candida albicans real time PCR Kit contains a specific ready-to-use system for the detection of Candida albicans through polymerase chain reaction (PCR) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the Candida albicans DNA. Fluorescence is emitted and measured by the real time systems´optical unit during the PCR. The detection of amplified fungi DNA fragment is performed in fluorimeter channel **FAM** with the fluorescent quencher **BHQ1**. DNA extraction buffer is available in the kit. In addition, the kit contains a system to identify possible PCR inhibition by measuring the **HEX/VIC/JOE** fluorescence of the internal control (IC).

4. Kit Contents

Ref.	Type of Reagent	Presentation 50rxns	
1	DNA Extraction Buffer	2 vials, 1.5ml	x2
2	CA Reaction Mix	1 vial, 950µl	x2
3	PCR Enzyme Mix	1 vial, 12µl	x2
4	Molecular Grade Water	1 vial, 400µl	x2
5	CA Internal Control	1 vial, 30µl	x2
6	CA Positive Control	1 vial, 30µl	x2

Analysis sensitivity: 5×10^3 copies/ml

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and Freezer
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks

7. ⚠ Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Quickly prepare the reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.

8. Sample Collection, Storage and transportation

- Collect samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is contained in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. You may use your own extraction systems or the commercial kit.

9.1.1 Sputum sample

1) Trypsin digestive Solution preparation: Add 10g trypsin to 200ml sterile purified water and mix thoroughly. Adjust the PH value to 8.0 with 2%NaOH solution. Add 2mL 25mmol/L $CaCl_2$, mix thoroughly and store at 4℃. Please incubate at 37℃ for 10 minutes before use. **2)** Estimate the volume of the sputum and add partes aequales of the Trypsin digestive Solution then vortex vigorously. Set at room temperature for 30 minutes. Transfer 0.5ml mixture to a new tube. Centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet. **3)** Add 1.0ml normal saline. Resuspend the pellet with vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet. **4)** Repeat step 3) **5)** Add 100µl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge. **6)** Incubate the tube for 10 minutes at 100°C. **7)** Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains DNA extracted and can be used for PCR template.

9.1.2 Swab sample

1) Wash the sample in 1ml normal saline and vortex vigorously. Transfer the liquid into anther tube, centrifuge at 13000rpm for 5 minutes. Keep the pellet by discarding the supernatant. **2)** Wash the sample in 1ml normal saline and vortex vigorously once more. Centrifuge at 13000rpm for 5 minutes. Keep the pellet by discarding the supernatant. **3)** Add 100µl DNA extraction buffer to the tube, closed the tube then vortex for 10 seconds. **4)** Incubation the tube for 10 minutes at 100℃. **5)** Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the extracted DNA and can be used for the template of the PCR.

9.1.3 Urine sample

1) Take 1ml sample, and centrifuge at 13000rpm for 5 minutes. Keep the pellet by discarding the supernatant. **2)** Wash the sample in 1ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Keep the pellet by discarding the supernatant. **3)** Add 100µl DNA extraction buffer to the tube, closed the tube then vortex for 10 seconds. **4)** Incubation the tube for 10 minutes at 100℃. **5)** Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the extracted DNA and can be used for the template of the PCR.

Attention:

A. During the incubation, make sure the tube is not open, as the vapor will volatilize into the air and may cause contamination if the sample is positive.

B. The extraction sample should be used in 3 hours or store at -20℃ for one month.

C. Different brand DNA extraction kits are available. You can also use your own extraction systems or the commercial kit based on the yield. For the DNA extraction, please comply with the manufacturer's instructions.

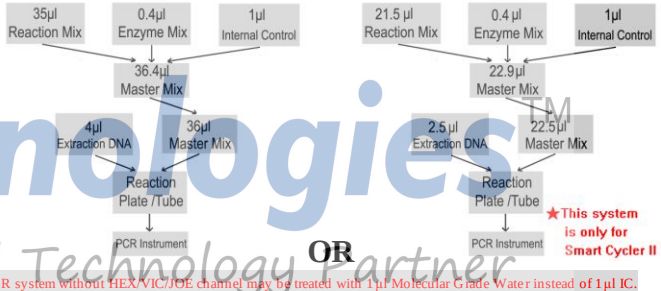
9.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal control (IC) allows the user to determine and control the possibility of PCR inhibition.

Add the internal control (IC) 1µl/rxn and the result will be shown in the HEX/VIC/JOE.

9.3 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



※PCR system without HEX/VIC/JOE channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of the controls and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **36µl (22.5µl for SmartCycer II)** Master Mix with micropipets of sterile filter tips to each real time PCR reaction plate/tube. Then separately add **4µl (2.5µl for SmartCycer II)** DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

37°C for 2min	1cycle
94°C for 2min	1cycle
93°C for 15sec, 60°C for 1min (Fluorescence measured at 60°C)	40cycles

Selection of fluorescence channels	
FAM	Target Nucleic Acid
HEX/VIC/JOE	IC

5) ⚠ If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**

10. Threshold setting: just above the maximum level of molecular grade water.

11. Quality control: Negative control, positive control and internal control must be performed correctly, otherwise the sample results is invalid.

Control \ Channel	Ct Value	
	FAM	HEX/VIC/JOE
Molecular Grade Water	UNDET	25~35
Positive Control(qualitative assay)	≤35	——

12. Data Analysis and Interpretation

The following sample results are possible:

	Ct Value		Result Analysis
	FAM	HEX/VIC/JOE	
1#	UNDET	25~35	Below the detection limit or negative
2#	≤38	——	Positive
3#	38~40	25~35	Re-test; if it is still 38~40, report as 1#
4#	UNDET	UNDET	PCR Inhibition; no diagnosis can be concluded.