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HIMEDIA®

MBPCR052 Hi-PCR® Candida albicans Semi-Q PCR Kit

Description

Candidiasis is a fungal infection caused by yeasts that belong to the genus *Candida*. There are over 20 species of *Candida* yeasts that can cause infection in humans, the most common of which is *Candida albicans*. *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. *C. albicans* is commensal and a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract. Overgrowth of the fungus results in candidiasis (candidosis). Candida can be easily managed. However it becomes more serious in immunocompromised patients, such as those with cancer, serious burns, or AIDS, where infection by Candida of internal organs can occur.

C. albicans normal habitat is the mucosal membranes of humans and various other mammals including the mouth, gut, vagina, and sometimes the skin. With healthcare becoming increasingly available to a large number of people, there is a greater need for faster molecular detection techniques as the number of samples have increased significantly. There is also a dire need of a detection technique which would reduce the time of assays from hours to mere minutes along with giving better sensitivity and specificity as compared to the current rapid detection tests.

The Hi-PCR® Candida albicans Semi-Q PCR Kit has been developed for rapid and highly sensitive detection of *C. albicans* using Semi Quantitative PCR systems.

NOTE: HiMedia's Hi-PCR® Candida albicans Semi-Q PCR Kit is for *in-vitro* use only.

Intended Use:

The Hi-PCR® Candida albicans Semi-Q PCR Kit is designed for fast detection of specific gene for *Candida albicans* from various cells, environmental sample, fecal samples and clinical material samples.

Principle:

The Hi-PCR® Candida albicans Semi-Q PCR Kit is designed for fast detection of specific sequence of **28S rRNA gene (175bp)** gene for *Candida albicans* using specific primers. The amplified target is detected by using agarose gel electrophoresis. This kit also contains **Internal control** and **Positive control.**

Internal control: This is a control sequence which is amplified in the same reaction tube along with the target sequence (target pathogen) but detected with a different primer (i.e. Multiplex PCR). An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

Positive control: This is a control reaction using a known template (target pathogen). A positive control is usually used to check that the primers have been designed properly and the PCR conditions have been set up correctly.
Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at a high temperature (Denaturation).

ISO 9001-2015 CERTIFIED WHO GMP ISO 13485-2016 CERTIFIED CE

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Sequence-specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). Taq DNA Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated between 25 to 40 times in each PCR assay.

Gel electrophoresis is used to analyze the amplification of desired gene region for target pathogen based on separation of DNA fragments according to their size.

Features:

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Sample source: Cells, environmental sample, fecal samples and clinical material samples

Storage:

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents should be avoided as this may reduce the sensitivity. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce sensitivity of the assay. HiMedia does not recommend using the kit after the expiry date stated on pack.

Kit Contents:

The provided PCR contains:

Components	Product codes	Reagents provided for (reactions)*	
		10R	**50R**
2X PCR TaqMixture	MBT061	300 µL	1.5 mL
Primer Mix for *Candida albicans*	DS0154	25 µL	125 µL
Primer Mix for Internal Control (685 bp)	DS0354	25 µL	125 µL
Molecular Biology Grade Water for PCR	ML065	500 µL	2.5 mL
6X Gel Loading Buffer	ML015	40 µL	200 µL
100 bp DNA Ladder	MBT049	30 µL	150 µL
Positive control (*Candida albicans* DNA)	DS0309	15 µL	75 µL
Internal Control DNA	DS0123	15 µL	75 µL

***** For a 50µl PCR reaction

Specimen collection and Handling:

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred in individual safety data sheets.

Sample Preparation:

Various food source samples, environmental samples, clinical materials, cultured bacteria and human fecal specimens can be examined. For preparation of Fungal DNA perform the nucleic acid purification using HiPurA® Fungal DNA Purification Kit (MB543) as described in the protocol.

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19)
- Thermal Cycler (Product Code: LA948/LA949/LA950/LA1006/LA1015/LA1059/LA1060/LA1066)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

General Preparation Instructions:

- Before use, suitable amount of all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control material (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

A. Protocol for PCR Master Mix Preparation

Perform PCR reactions for each DNA sample as per the following table:

Components	Recommended volume to be added per reaction (µL)
2X PCR TaqMixture **(MBT061)**	25 µL
Primer Mix for *Candida albicans* **(DS0154)**	2 µL
Primer Mix for Internal Control (685 bp) **(DS0354)**	2 µL
Template DNA	2 µL
Internal Control DNA **(DS0123)**	1 µL
Molecular Biology Grade Water for PCR **(ML065)**	Up to 50 µL

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 1µL of Positive control DNA (provided) in a separate tube.

Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Interpret the data using Agarose Gel Electrophoresis.

B. R**ecommended PCR program:**

1	Initial denaturation	94°C for 5 minutes	No. of cycles: 1
2	Denaturation	94°C for 45 seconds	No. of cycles: 30
3	Annealing	56°C for 30 seconds	
4	Extension	72°C for 30 seconds	
5	Final Extension	72°C for 05 minutes	No. of cycles: 1

After amplification, the products can be kept at 4°C overnight or frozen at –20°C for long-term storage.

PCR Assay Results Interpretation:

- For analysis of the PCR data, load 10 µl of amplicon on a 1.5% Agarose gel along with 1 µl of 6X Gel Loading Buffer (ML015).
- Load 3 µl of 100 bp DNA ladder (MBT049) in separate well.

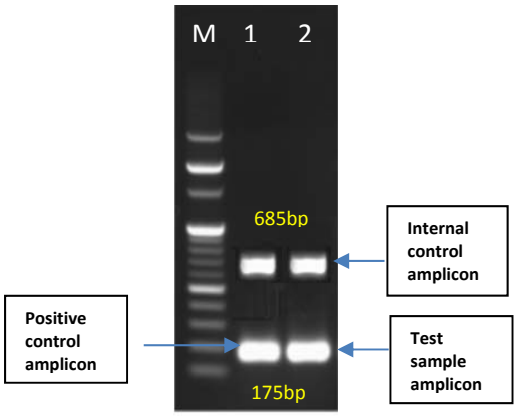
EtBr-staining to check results:

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 min.
- Confirm the expected amplicon size comparing with 100 bp DNA marker.

Quality Control:

Each lot of HiMedia's Hi-PCR® Candida albicans Semi-Q PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification

Amplification Data:



Lane no.	Samples
1	100 bp DNA ladder
2	Positive control amplicon of *Candida albicans* (175bp) with internal control (685 bp)
3	Test sample amplicon of *Candida albicans* (175bp) with internal control (685 bp)

Figure: Gel image representing amplification of 28S rRNA gene using target sample of *C. albicans* with positive control (175bp) and internal control (685bp)

Warning

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use.

Precautions

Read the procedure carefully before starting the experiment. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation:

Each lot of HiMedia's Hi-PCR® Candida albicans Semi-Q PCR Kit is tested against predetermined specifications to ensure consistent product quality

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1	No amplification	Degraded samples	Check the integrity of DNA using agarose gel electrophoresis.
			Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Impure DNA	Ensure that the DNA is free of any Impurities. The DNA purity should be between 1.6-1.8.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

The Hi-PCR® Candida albicans Semi-Q PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
	Do not use if package is damaged
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Disclaimer :

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