

## LightMix<sup>®</sup> Kit *Candida albicans* Cat.-No. 40-0591-32

Kit with reagents for the detection of *Candida albicans* DNA using the Roche Diagnostics LightCycler<sup>®</sup> 1.x / 2.0 / 480 / 480II Instruments.

Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each.  
**Store protected from light at room temperature (18-25°C), do NOT freeze!**

Instructions for use with the LightCycler<sup>®</sup> 1.x / 2.0 Instruments see pages 4-5  
Instructions for use with the LightCycler<sup>®</sup> 480 / 480II Instruments see pages 6-7

### 1. Introduction

Fungi are ubiquitous microorganisms best known from moulded food. *Candida albicans* is a common yeast species and part of the normal intestinal flora in most humans, but is also known to cause opportunistic oral and genital infections. Immunocompromised individuals such as leukemic patients, solid organ recipients and HIV-1 patients suffer from invasive fungal infections. An early diagnosis is crucial for a successful antifungal therapy.

Conventional diagnostic tests by blood culture are slow and have been reported to lack sufficient sensitivity and specificity while PCR has been stated to yield better results<sup>1</sup> for an early diagnosis.

This LightMix<sup>®</sup> Kit contains primers and hybridization probes targeting the 18S RNA genes as published<sup>1,2</sup>.

The has been tested with the Roche Diagnostics "LightCycler<sup>®</sup> FastStart DNA Master Hybridization Probes" ready-to-use reaction mix in the LightCycler<sup>®</sup> Instrument 2.0.

<sup>1</sup> Quantification of Fungal DNA Using Fluorescence Resonance Energy Transfer and the LightCycler System., Löffler J, Henke N, Hebart H, Schmidt D, Hagemeyer L, Schumacher U, Einsele H. JCM 38 (2000) 586-590

<sup>3</sup> Molecular detection and identification of *Candida* and *Aspergillus* sp p. from clinical samples using real-time PCR. Klingspor L. and Jalal S. Clin Microbiol Infect 2006; 12: 745-753

The LightMix<sup>®</sup> Kit *Candida albicans* provides a fast, easy and accurate system to identify this target in a nucleic acid extract.

This LightMix<sup>®</sup> Kit is tested on the LightCycler<sup>®</sup> 1.x / 2.0 Instruments with Roche Diagnostics 'LightCycler<sup>®</sup> FastStart DNA Master HybProbe'. On the LightCycler<sup>®</sup> 480 Instruments (96 well and 384 well formats) it is tested with Roche Diagnostics 'LightCycler<sup>®</sup> FastStart DNA Master HybProbe'.

### 2. Description

The LightMix<sup>®</sup> Kit detects a region of the fungal 18S RNA gene indicating the presence of fungal DNA in a nucleic acid extract. As *Candida* is part of the normal human flora this kit can yield false positive results due to laboratory contaminations.

A 490 bp long fragment of the *Candida albicans* genome is amplified with primers and detected with specific probes labeled with LightCycler<sup>®</sup> Red 640 (detected in channel 640).

For use in LightCycler<sup>®</sup> 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler<sup>®</sup> 1.x Instruments to software version 4.1.

### 3. Set contents

- 3 Vials with green caps containing premixed lyophilized primers and probes for 32 PCR reactions each of *Candida albicans*
- 1 Vial with colorless cap containing control DNA *Candida albicans* , 10<sup>5</sup> target equivalents per reaction

### 4. Additional reagents and items required

*Roche Diagnostics:*

LightCycler® FastStart DNA Master HybProbe	Cat.-No. 03 003 248 001
High Pure PCR Template Preparation Kit	Cat.-No. 11 796 828 001
LightCycler® Capillaries (20 µl) (LightCycler® 1.x / 2.0 Instruments)	Cat.-No. 04 929 292 001
LightCycler® 480 Multiwell Plate 384, white (LightCycler® 480 Instrument)	Cat.-No. 04 729 749 001
or LightCycler® 480 Multiwell Plate 96, white (LightCycler® 480 Instrument)	Cat.-No. 04 729 692 001

### 5. Product characteristics

PCR results are obtained within 60 minutes (45 cycles and melting curve) with the LightCycler® 1.x / 2.0 Instruments and within 75 minutes (50 cycles and melting curve) with the LightCycler® 480 Instruments.

#### Sensitivity

These reagents detect 10 copies of *Candida albicans* DNA using the Roche 'LightCycler® FastStart DNA Master HybProbe' with the LightCycler® 1.x / 2.0 / 480 Instruments. (in an exemplary system, using cloned targets as reference).

#### Measuring range

The linear measuring range of the assay is 10<sup>2</sup> to 10<sup>6</sup> copies of *Candida albicans* DNA using the Roche 'LightCycler® FastStart DNA Master HybProbe' with the LightCycler® 1.x / 2.0 / 480 Instruments.

#### Storage and Stability

- Lyophilized reagents are stable for at least 3 months after shipment when stored protected from light at room temperature (18-25°C).
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 5 days when stored protected from light and refrigerated (4°C).

## 6. Experimental protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 / 480 Instruments. Start programming before preparing the solutions. See the LightCycler® Instrument operator's manual for details.

**Sample material:** Use aqueous nucleic acid preparations (e.g. Roche Diagnostics 'High Pure PCR Template Preparation Kit').

**Negative control:** Always run at least one no-template control (NTC) - replace the template DNA with water.

**Positive control:** Run a positive control - replace the template DNA with the provided control DNA.

### 6.1. Preparation of parameter-specific reagents (32 reactions):

One reagent vial with a **green** cap contains primers and probes to run 32 LightCycler® reactions for *Candida albicans*

**Add 66 µl** PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

► **Use 2 µl** reagent for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C. Avoid prolonged exposure to light.

### 6.2. Preparation of the control DNA

**Add 40 µl** PCR-grade water to the vial ( $8 \times 10^5$  target molecules) with a colorless cap. Mix the target DNA by pipetting the solution up and down 10 times (final concentration:  $10^5$  target molecules in 5 µl).

► **Use 5 µl** control DNA for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C, for long term storage freeze at -20°C. Avoid repeated freezing thawing cycles. For the control DNA provided with the kit please note that the relative amounts of DNA may change during time.

| Please note that opening this vial may cause contaminations of the work-space (aerosol).

### 6.3. Preparation of the LightCycler® reaction mix

In a cooled reaction tube, prepare the reaction mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

For use with the Roche FastStart Master	
Single reaction	Component
9.4 µl	water, PCR-grade (colorless cap, provided with the Roche Master kit)
1.6 µl	Mg <sup>2+</sup> solution 25 mM (blue cap, provided with the Roche FastStart kit)
2.0 µl	<b>reagent</b> mix (parameter specific reagents containing primers and probes, see 6.1.)
2.0 µl	Roche Master (red cap, for preparation see Roche manual)

**15.0 µl**

Volume of reaction mix

Mix gently, spin down and **transfer 15 µl** each of the reaction mix to a LightCycler® capillary (LightCycler® 1.x / 2.0 Instrument) or to a multiwell plate (LightCycler® 480 Instruments).

**Add 5 µl** of sample or control DNA to each capillary or well for a final reaction volume of 20 µl.

Start run.

## 7. LightCycler® 1.x / 2.0 Instruments

### 7.1. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:	Denaturation	Cycling			Melting			Cooling
<b>Parameter</b>								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	45			1			1
Target [°C]	95	95	62	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:15	00:00:25	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]	20	20	20	20	20	20	0.2	20
Acquisition Mode	None	None	Single	None	None	None	Continuous	None

### 7.2. Data Analysis

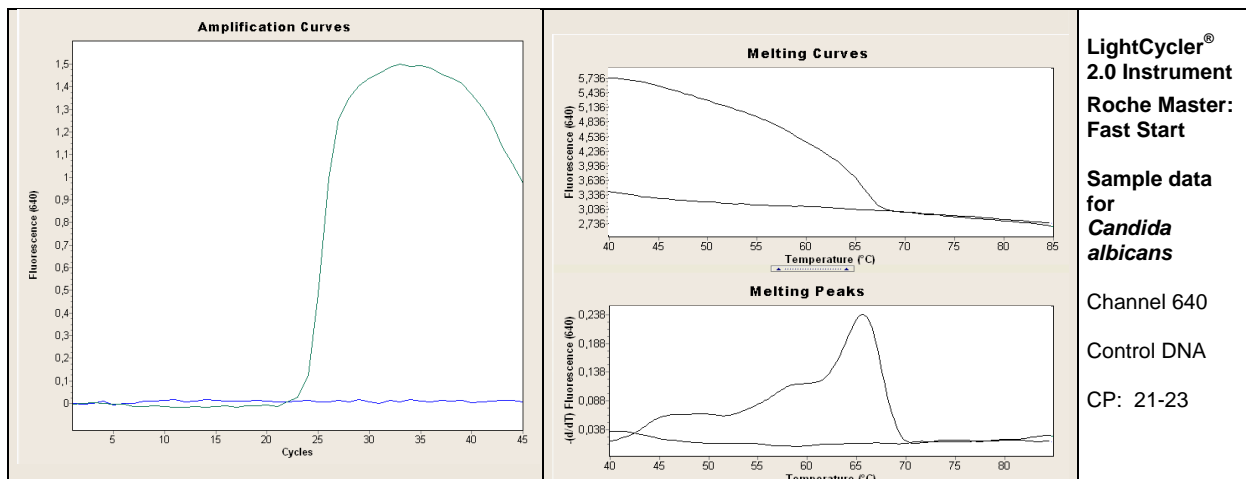
For use in LightCycler® 1.x Instruments select channel F2 instead of channel 640 and channel F1 instead of channel 530 for detection.

Perform data analysis, as described in the LightCycler® Instrument operator's manual.

We recommend using the Second Derivative Maximum method (Automated (F'' max)). The cycle number of the Crossing Point (CP) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *Candida albicans* data in channel 640, Quantification mode (LightCycler® 2.0 Instrument). The negative control (NTC) must show no signal. For the identification of the PCR product view *Candida albicans* data in channel 640, Melting Curves mode.

### 7.3. Sample Data – typical results



**Fig.1. Sample data for the *Candida albicans* detection system.**

**Upper panels:** Data from LightCycler® 2.0 Instrument. Left panel channel 640 quantification mode (Second Derivative Maximum) with amplification curves for *Candida albicans* Right panel channel 640 melting analysis for *Candida albicans* (not relevant for detection).

**\* Note:** Fluorescence levels depend on instrument settings and may vary. The characteristics of the curve (shape and trend of fluorescence levels) should be similar to the curve in the manual. The fluorescent curves over cycles (quantification mode) must be smooth and not zigzag shaped. The CP values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (delta CP). The CP values described in this manual (chart text) have been obtained using the supplied standard row; the entire set of curves can be horizontal shifted.

### 7.4. Interpretation of data

Candida albicans (sample)	NTC	Result
no amplification	negative	Negative
amplification signal	negative	Positive
no amplification	not relevant	PCR failure, repeat experiment
amplification signal	positive	Contamination, repeat experiment

#### Typical analysis results (LightCycler® 2.0 Instrument, Roche Master: Fast Start)

**Notes:** The values of the respective melting temperatures ( $T_M$ ) may vary  $\pm 2.5^\circ\text{C}$  between different experiments. The  $\Delta T$  between the melting peaks for heterozygote genotypes may vary  $\pm 1.5^\circ\text{C}$ . Samples with deviating melting curves should be subject to further investigations; sequence analysis can be provided by TIB MOLBIOL Berlin (contact [service@tib-molbiol.de](mailto:service@tib-molbiol.de)).

#### Warning:

*Candida* is part of the normal human flora, increasing the potential for low false positives results even in a very clean diagnostic laboratory environment. Repeat the analysis for low-positive samples reporting 10 or less copies per reaction.

## 8. LightCycler® 480 Instrument

### 8.1. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Detection Format:

LightCycler® 480 Instrument: 483-640

LightCycler® 480 II Instrument: 498-640

Program Step:	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	50			1			1
Target [°C]	95	95	60	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:15	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate [°C/s] 96	4.4	4.4	2.2	4.4	4.4	1.5	-	1.5
Ramp Rate [°C/s] 384	4.6	4.6	2.4	4.6	4.6	2.0	-	2.0
Acquisition Mode	None	None	Single	None	None	None	Continuous	None
Acquisitions [per °C]	-	-	-	-	-	-	3	-

(Melting not relevant for detection)

### 8.2. Data Analysis

Note: For use on LightCycler® 480 II Instruments select Filter Combination 498-640 instead of Filter Combination 483-640 for detection.

Perform data analysis, as described in the LightCycler® Instrument operator's manual.

We recommend using the Second Derivative Maximum method (Automated (F'' max)). The cycle number of the Crossing Point (CP) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *Candida albicans* data with Filter Combination 483-640 Quantification mode. The negative control (NTC) must show no signal. For the identification of the PCR product view *Candida albicans* data with Filter Combination 483-640, Melting Curves mode.

### 8.3. Sample Data – typical results

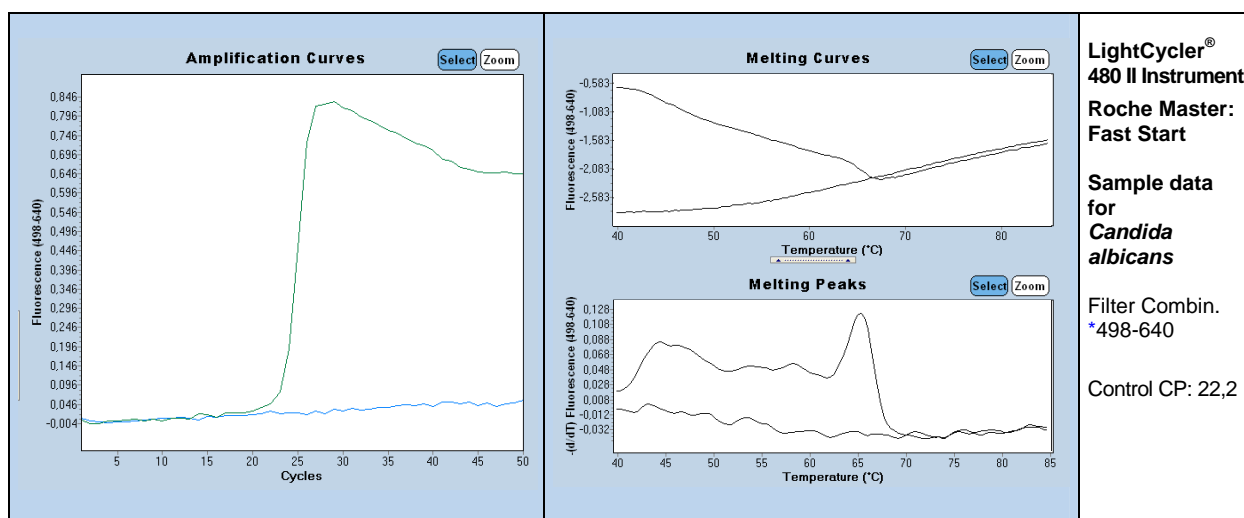


Fig.1. Sample data for the *Candida albicans* detection system.

**Upper panels:** Data from LightCycler® 480 II Instrument. Left panel Filter Combination 498-640 quantification mode (Second Derivative Maximum) with amplification curves for *Candida albicans*. Right panel Filter Combination 498-640 melting analysis for *Candida albicans* (not relevant for detection).

\* **Note:** Fluorescence levels depend on instrument settings and may vary. The characteristics of the curve (shape and trend of fluorescence levels) should be similar to the curve in the manual. The fluorescent curves over cycles (quantification mode) must be smooth and not zigzag shaped. The CP values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (delta CP). The CP values described in this manual (chart text) have been obtained using the supplied standard row; the entire set of curves can be horizontal shifted.

### 8.4. Interpretation of data

Candida albicans (sample)	NTC	Result
no amplification	negative	Negative
amplification signal	negative	Positive
no amplification	not relevant	PCR failure, repeat
amplification signal	positive	Contamination, repeat

#### Typical analysis results (LightCycler® 480 II Instrument, Roche Master: Fast Start)

**Notes:** The values of the respective melting temperatures ( $T_m$ ) may vary  $\pm 2.5^\circ\text{C}$  between different experiments. The  $\Delta T$  between the melting peaks for heterozygote genotypes may vary  $\pm 1.5^\circ\text{C}$ . Samples with deviating melting curves should be subject to further investigations; sequence analysis can be provided by TIB MOLBIOL Berlin (contact [service@tib-molbiol.de](mailto:service@tib-molbiol.de)).

#### Warning:

*Candida* is part of the normal human flora, increasing the potential for low false positives results even in a very clean diagnostic laboratory environment. Repeat the analysis for low-positive samples reporting 10 or less copies per reaction.

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These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany.  
LightCycler<sup>®</sup> hybridization probes produced under license from Roche Diagnostics GmbH.

