



For *in Vitro* Diagnostic Use



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# AmpliSens® *Candida albicans*-FEP PCR kit

## Instruction Manual



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### 1. INTENDED USE.

**AmpliSens® *Candida albicans*-FEP** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Candida albicans* DNA in the clinical material (cervical, urethral scrapes (swabs); urine sediment) by using end-point hybridization-fluorescence detection of amplified products.

### 2. PRINCIPLE OF PCR DETECTION.

*Candida albicans* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Candida albicans* primers. In Fluorescent End-Point PCR, the amplified product is detected by using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens® *Candida albicans*-FEP** PCR is a qualitative test, which contains the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *Candida albicans*-FEP** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer or chemically modified polymerase (TaqF). Wax melting and reaction mix components occur only at 95°C. Chemically modified polymerase (TaqF) is activated by heating at 95°C for 15 min.

### 3. CONTENTS OF THE KIT.

**AmpliSens® *Candida albicans*-FEP** PCR kit is produced in 2 forms:

AmpliSens® *Candida albicans*-FEP PCR kit (tubes of 0.5 ml volume), **REF** F1-100-R0,5-FEP-CE.

AmpliSens® *Candida albicans*-FEP PCR kit (tubes of 0.2 ml volume), **REF** F1-100-R0,2-FEP-CE.

**AmpliSens® *Candida albicans*-FEP** PCR kit includes:

Reagent	Description	Volume (ml)	Quantity
<b>PCR-mix-1-FEP/FRT <i>Candida albicans</i></b> ready-to-use single-dose test tubes ( <i>under wax</i> )	colorless clear liquid	0.008	110 tubes of 0.5 or 0.2 ml
<b>PCR-mix-2-FL</b>	colorless clear liquid	0.77	1 tube
<b>Mineral oil for PCR</b>	colorless viscous liquid	4.0	1 dropper bottle
<b>PCR-mix-Background</b>	colorless clear liquid	0.5	1 tube
<b>Positive Control DNA <i>Candida albicans</i> (C+)</b>	colorless clear liquid	0.2	1 tube
<b>DNA-buffer</b>	colorless clear liquid	0.5	1 tube
<b>Negative Control (C-)*</b>	colorless clear liquid	1.2	1 tube
<b>Internal Control-FL (IC)**</b>	colorless clear liquid	1.0	1 tube

\* must be used in the isolation procedure as Negative Control of Extraction.

\*\* add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture (see “DNA-sorb-AM” **REF** K1-12-100-CE protocol).

**AmpliSens® *Candida albicans*-FEP** PCR kit is intended for 110 reactions, including controls.

### 4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit
- Disposable powder-free gloves and laboratory coat
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters (up to 200 µl)
- Tube racks
- Vortex mixer
- Desktop centrifuge with rotor
- PCR box
- Personal thermocycler (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 or GeneAmp PCR System 2400 (Applied Biosystems), Uno-2 (Biometra) or equivalent)
- Fluorometer ALA-1/4 (“Biosan”, Latvia) or equivalent
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator for temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus16 °C.
- Waste bin for used tips.

### 5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.

- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional, it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.



Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

## 6. SAMPLING AND HANDLING.



Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

**AmpliSens® *Candida albicans*-FEP** PCR kit is intended for analysis of DNA extracted by using DNA isolation kits from urogenital tract mucous membranes scrapes (swabs); urine sediment (first portion of the morning specimen).

## 7. PROTOCOL.

### 7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-AM", **REF** K1-12-100-CE.



Please carry out the DNA isolation according to the manufacturer instruction.

### 7.2. Preparing the PCR.

Total reaction volume - **25 µl**, volume of DNA sample - **10 µl**.

#### 7.2.1 Preparing tubes for PCR.

1. Prepare the required quantity of tubes with **PCR-mix-1-FEP/FRT *Candida albicans*** and wax for amplification of DNA from clinical and control samples.

2. Add **7 µl** of **PCR-mix-2-FL** to the surface of wax layer of each tube, ensuring that it does not fall under the wax and mix with PCR-mix-1-FEP/FRT *Candida albicans*.
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**).
4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT *Candida albicans*** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of wax layer of each tube, ensuring that it does not fall under the wax and mix with PCR-mix-1-FEP/FRT *Candida albicans*. Add above **1 drop** of **mineral oil for PCR**.



Background samples, that have once passed thermocycling, can be used for further runs without thermo cycling. Multiple use of Background samples is permitted only if the PCR kit of the same lot is applied. Store the Background tubes at 2-25 °C for 1 week. Keep away from light.

5. Using tips with aerosol filter add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction.



The tubes with PCR-mix-1-FEP/FRT *Candida albicans* that are not used at the moment should be kept away from light.

6. Carry out the control amplification reactions:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ - Add **10 µl** of **Positive Control *Candida albicans*** to the tube labeled C+ (Positive Control of Amplification).

#### 7.2.2 Amplification.

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95°C (pause regimen), insert tubes into the cells of amplifier and press the button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before their insertion in a thermocycler.

Table 1

Programming thermocyclers at DNA amplification of *Candida albicans* (65-60-45)

Step	Thermocyclers with active temperature adjustment:									Thermocyclers with block temperature adjustment:		
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (Applied Biosystems)			"Gradient Palm Cycler" (Corbett Research),			"Uno-2" (Biometra)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	2 sec	35	95 °C	20 sec	20	95 °C	2 sec	24	95 °C	25 sec	20
	65 °C	5 sec		65 °C	25 sec		65 °C	10 sec		65 °C	40 sec	
	72 °C	5 sec		72 °C	30 sec		72 °C	10 sec		72 °C	40 sec	
3	95 °C	2 sec	9	95 °C	20 sec	24	95 °C	2 sec	19	95 °C	25 sec	24
	60 °C	10 sec		60 °C	30 sec		60 °C	15 sec		60 °C	40 sec	
	72 °C	5 sec		72 °C	30 sec		72 °C	10 sec		72 °C	40 sec	
4	95 °C	2 sec	1	95 °C	20 sec	1	95 °C	2 sec	1	95 °C	25 sec	1
	60 °C	10 sec		60 °C	30 sec		60 °C	15 sec		60 °C	40 sec	
5	10 °C	storage		10 °C	storage		10 °C	storage		10 °C	storage	

## 8. DATA ANALYSIS.

Detection is conducted on ALA-1/4 fluorescence detector.



Please read Aladin Operating Manual before use of this kit.

Program the detector according to manufacturer's manual and Appendix 1.



Detection can be conducted within 1 day from the end of the amplification only if the tubes with amplified product were stored at 28°C and below in a light-protected place.

### Results interpretation

1. When the analysis is complete the results are automatically shown in the table in the manner of following indications:

**pos** – positive result;

**neg** – negative result;

**eq** – equivocal result (signal is in grey zone);

**nd** – invalid result (specific signal and IC signal are absent in the sample).

2. Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (see Table 2).

Table 2

Results for controls

Control	Stage for control	Results		Interpretation
		FAM channel (samples)	HEX channel (IC)	
C-	DNA isolation	<i>Candida albicans</i> - neg	+	OK
NCA	Amplification	<i>Candida albicans</i> - nd	-	OK
C+	Amplification	<i>Candida albicans</i> - pos	-	OK

## 9. TROUBLESHOOTING.

Results of analysis are not being registered in the following cases:

- No positive signal with positive control of PCR (C+) can indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or storage conditions for kit components did not comply with manufacturer instruction, or reagents kit has expired. It is necessary to check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
- If no signal was detected in both channels, for detection of pathogen DNA and for detection of Internal Control, the sample should be examined repeatedly (PCR and detection). The same procedures should be applied to the samples with equivocal result, that is, the specific signal exceeds the background not enough to consider the sample as positive. If equivocal result is registered in the second run, the

analysis should be repeated starting from the stage of DNA extraction.

- Positive signal in negative controls (NCE, C-) indicates the reagents or samples contamination. In such case results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting of contamination source must be undertaken.

If you have any further questions or if encounter problems, please contact our Authorized representative in the European Community.

## 10. STABILITY AND STORAGE.

All components of the **AmpliSens® *Candida albicans*-FEP** are to be stored at the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens® *Candida albicans* -FEP** PCR kit are to be stable until labeled expiration date.

## 11. SPECIFICATIONS.

### 11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® *Candida albicans*-FEP** PCR kit is no less than 1x10<sup>4</sup> genome equivalents per 1 ml of sample (GE/ml).



Claimed analytical features of **AmpliSens® *Candida albicans*-FEP** PCR kit are guaranteed only when additional reagents kits, "DNA-sorb-AM" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

### 11.2. Specificity.

Specificity of **AmpliSens® *Candida albicans*-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® *Candida albicans*-FEP** PCR kit was confirmed in laboratory clinical trials.












## 12. REFERENCES.

1. Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

## 13. QUALITY CONTROL.

In compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Quality Management System, each lot of **AmpliSens® *Candida albicans*-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number	IC	Internal Control
	Contains sufficient for <n> tests		Authorised representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
C+	Positive Control	C-	Negative control
NCA	Negative Control of Amplification		