



For Professional Use Only

AmpliSens[®] *Candida albicans*-EPh

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE

AmpliSens® *Candida albicans*-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Candida albicans* in the clinical material (cervical or urethral scrapes (swabs) by using electrophoretic detection of the amplified products in agarose gel.



The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Candida albicans detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *Candida albicans* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® *Candida albicans*-EPh PCR kit** PCR kit is a qualitative test, which contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *Influenza virus A/B*-EPh 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. Wax melting and reaction mix components occur only at 95 °C.

3. CONTENT

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

AmpliSens® *Candida albicans*-EPh PCR kit variant 100 R (tubes 0.5 ml),
REF F1-100-R0,5-CE.

AmpliSens® *Candida albicans*-EPh PCR kit variant 100 R (tubes 0.2 ml),
REF F1-100-R0,2-CE.

AmpliSens® *Candida albicans*-EPh PCR kit variant 100 R includes:

Reagent	Description	Volume, ml	Amount
PCR-mix -1-R <i>Candida albicans</i> ready-to-use single-dose test tubes (<i>under wax</i>)	colorless clear liquid	0.005	110 tubes of 0.5 or 0.2 ml
PCR-mix-2 blue	blue clear liquid of color	1.2	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
Positive Control DNA <i>Candida albicans</i> (C+<i>C.albicans</i>)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	1 tube
Internal Control complex (ICc)**	colorless clear liquid	1.0	1 tube

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

** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-7-100-CE protocol).

AmpliSens® *Candida albicans*-EPh PCR kit variant 100 R is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems), Uno-2 (Biometra), MiniCycler, PTC-100 (MJ Research), Terzik (DNA-Technology)).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °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 are described in manufacturer's handbook [1]. It is recommended to read this handbook before starting work.

AmpliSens[®] *Candida albicans*-EPh PCR kit is intended for analysis of DNA extracted with DNA extraction kits from:

- *Cervical or urethral scrapes (swabs).*



Only one freeze-thaw cycle of clinical material is allowed.

7. WORKING CONDITIONS

AmpliSens[®] *Candida albicans*-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

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

- DNA-sorb-AM, **REF** K1-7-100-CE.



Carry the RNA extraction in compliance with the manufacturer protocol.

8.2. Preparing the PCR.

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

8.2.1 Preparing tubes for PCR.

1. Collect the required number of tubes prepared as describes above or tubes with **PCR-mix-1-R *Candida albicans*** with wax for amplification of DNA from clinical and control samples.

2. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, so that it wouldn't fall under the wax and mix with reagents in the tube.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl). When using thermocycler with heating cover this step could be omitted.
4. Using tips with aerosol barrier add **10 µl of DNA samples** obtained from clinical or control samples.
5. 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 DNA *Candida albicans*** to the tube labeled C+*C. albicans* (Positive Control of Amplification).

8.2.2 Amplification.

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

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

Table 1

Programming thermocyclers for *Candida albicans* DNA amplification

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 II (Biometra), MiniCycler, PTC-100 (MJ Research)		
Step	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	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
2	95 °C	10 s	42	95 °C	15 s	42	95 °C	1 min	42
	65 °C	10 s		65 °C	25 s		65 °C	1 min	
	72 °C	10 s		72 °C	25 s		72 °C	1 min	
3	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
4	4 °C	storage		4 °C	storage		10 °C	storage	

Amplification in thermocycler with block temperature adjustment lasts 2 h 30 min, in thermocycler with active temperature adjustment — 1 h 50 min.

After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2–8 °C (be sure to warm the samples to room temperature before running electrophoresis).

9. DATA ANALYSIS

It's recommended to use the following detection agarose kit:

- EPh variant 200, **REF** K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7%). The length of specific amplified DNA fragments is:

- *Candida albicans* - 370 bp.
- Internal Control - 730 bp.



Put the protective mask or use the glass filter while watching and photographing the gel

9.1. Results interpretation

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel		Interpretation
		370 bp	730 bp	
C-	DNA extraction	No	Yes	OK
NCA	Amplification	No	No	OK
C+	Amplification	Yes	No	OK

- The sample is considered to be positive for *Candida albicans* DNA if the band of 370 bp is present in agarose gel. The band of ICc (730 bp) could be absent in the samples with high concentration of *Candida albicans* DNA.
- The sample is considered to be negative for *Candida albicans* DNA if the band of 370 bp is absent and the band of 730 bp is present.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

10. TROUBLESHOOTING

Analysis results are not obtained as per the following examples:

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests are to be re-installed. Discard any reagents that may be suspect.
- If in lanes none of bands of 370 and 730 nucleotide pairs is observed, result of analysis for this sample is irrelevant and investigation of this sample must be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.
- If in lines nonspecific bands at different levels are presented, it may be caused by lack of “hot start” or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C–) specific band of 370 bp appears it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

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

11. TRANSPORTATION

AmpliSens® *Candida albicans*-EPh PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of **AmpliSens® *Candida albicans*-EPh** PCR kit are to be stored at 2–8 °C when not in use. All components of the PCR kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *Candida albicans*-EPh** PCR kit is no less than 5×10^3 genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens® *Candida albicans*-EPh** PCR kit are guaranteed only when additional kits of reagents DNA-sorb-AM and EPh (manufactured by Federal Budget Institute of Science “Central Research Institute for Epidemiology”) are used.

13.2. Specificity

Specificity of **AmpliSens® *Candida albicans*-EPh** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.














14. REFERENCES.

1. Gottfredsson M, Cox GM, Perfect JR. Molecular methods for epidemiological and diagnostic studies of fungal infections. Pathology. 1998 Nov;30(4):405-18.
2. Manual “Sampling, transportation and storage of clinical material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”, Moscow, 2008.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Candida albicans*-EPh** PCR kit is tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

	Catalogue number		Sufficient for
	Batch code		Expiration Date
	<i>In vitro</i> diagnostic medical device		Consult instructions for use
	Version		Keep away from sunlight
	Temperature limitation	NCA	Negative control of amplification
	Manufacturer	C-	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
	Authorised representative in the European Community	ICc	Internal Control complex
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
12.11.10	Through the text	Records about PCR kit variant 200 are deleted
25.12.10 KM	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added.
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
Key to Symbols Used	The explanation of symbols was corrected	
15.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”