CE

IVD

For Professional Use Only

AmpliSens® *C.albicans / C.glabrata / C.krusei*-MULTIPRIME-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of Science “Central Research Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

1. INTENDED USE 3
2. PRINCIPLE OF PCR DETECTION 3
3. CONTENT 3
4. ADDITIONAL REQUIREMENTS 5
5. GENERAL PRECAUTIONS 5
6. SAMPLING AND HANDLING 6
7. WORKING CONDITIONS 6
8. PROTOCOL 6
9. DATA ANALYSIS 8
10. TROUBLESHOOTING 10
11. TRANSPORTATION 10
12. STABILITY AND STORAGE 10
14. REFERENCES 11
15. QUALITY CONTROL 12
16. KEY TO SYMBOLS USED 13

1. INTENDED USE

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of DNA of *Candida albicans, Candida glabrata,* and *Candida krusei* in the clinical material (urogenital, rectal, and pharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

C.albicans / *C.glabrata* / *C.krusei* DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *C.albicans* / *C.glabrata* / *C.krusei* primers. In real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit is a qualitative test that contains the Internal Control (**Internal Control-FL (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® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions In variant FRT “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using a wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 °C. The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit is produced in 2 forms:

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit variant FRT **REF** R-F3(RG)-CE.

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit variant FRT-100 F **REF** R-F3-F(RG,iQ)-CE.

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *C.albicans* / *C.glabrata* / *C.krusei* (ready-to-use single-dose test tubes (*under wax*))	clear liquid from colorless to light lilac colour	0.01	110 tubes of 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	1 tube
Positive Control complex (C+)	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-FL (IC)**	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-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture (see **DNA-sorb-AM**, **REF** K1-12-100-CE protocol).

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit is intended for 110 reactions (including controls).

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit variant FRT-100 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *C.albicans* / *C.glabrata* / *C.krusei*	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
Positive Control complex (C+)	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-FL (IC)**	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-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture (see **DNA-sorb-AM**, **REF** K1-12-100-CE protocol).

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- 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 a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany); iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA) or equivalent).
- Disposable polypropylene tubes when working with PCR kit variant FRT-100 F :
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  b) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- 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 regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, it should begin in the Extraction Area and then 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 the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the clinical material (urogenital, rectal, and pharyngeal swabs; conjunctival discharge; prostate gland secretion; urine samples (sediment of the first portion of the morning specimen)).

7. WORKING CONDITIONS

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- DNA-sorb-AM, **REF** K1-12-100-CE.
- For other nucleic acid extraction kits see Guidelines [2].

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL**

(IC).

In the extraction procedure it is necessary to carry out the control reactions as follows:

C–

– Add **100 µl of Negative Control (C–)** to the tube labeled C– (Negative Control of Extraction).

Extract the DNA according to the manufacturer's protocol.

8.2. Preparing PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

8.2.1. Preparing tubes for PCR

Variant FRT

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

1. Prepare the required number of tubes with **PCR-mix-1-FL *C.albicans* / *C.glabrata* / *C.krusei*** and wax for amplification of DNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2-FL-red** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *C.albicans* / *C.glabrata* / *C.krusei***.

Variant FRT-100F

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

1. Thaw the required number of tubes with **PCR-mix-2-FRT**. Vortex the tubes with **PCR-mix-1-FL *C.albicans* / *C.glabrata* / *C.krusei*, PCR-mix-2-FRT, and polymerase (TaqF)** and sediment the drops by short centrifugation (1-2 s).

   Take the required number of tubes/strips for amplification of the cDNA obtained from clinical and control samples.
2. For N reactions (including 2 controls), add to a new tube:
   – **10*(N+1) µl** of **PCR-mix-1-FL *C.albicans* / *C.glabrata* / *C.krusei***,
   – **5.0*(N+1) µl** of **PCR-mix-2-FRT**,
   – **0.5*(N+1) µl** of **polymerase (TaqF)**.

   Mix the prepared mixture and sediment the drops by short centrifugation (1-2 s). Transfer **15 µl** of the prepared mixture to prepared tubes.

Steps 3 and 4 are required in both variants.

3. Add **10 µl** of **DNA** obtained at the DNA extraction stage to the prepared tubes.
4. 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 complex (C+)** to the tube labeled C+ (Positive Control of Amplification).

C– – Add 10 µl of sample extracted from the **Negative Control (C–)** reagent to the tube labeled C– (Negative Control of Extraction).

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

1. Create a temperature profile on your instrument as follows:

Table 1

AmpliSens-1 amplification program

	Rotor-type Instruments[1]			Plate-type Instruments[2]		
Step	***Temperature, °C***	***Time***	***Cycles***	***Temperature, °C***	***Time***	***Cycles***
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s Fluorescence acquiring		**60**	30 s Fluorescence acquiring	
	72	15 s		**72**	15 s	

Fluorescent signal is detected in the channels for the FAM, JOE, ROX, and Cy5 fluorophores. Other channels are enabled if several tests are simultaneously carried out in a single run.

2. Adjust the fluorescence channel sensitivity according to Important Product Information *Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in four channels:

– The signal of the *Candida albicans* DNA amplification product is detected in the channel for the FAM fluorophore.

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[2] For example, iQ5, Mx3000P, Mx3000, DT-96 or equivalent.

– The signal of the *Candida glabrata* DNA amplification product is detected in the channel for the JOE fluorophore.
– The signal of the *Candida krusei* DNA amplification product is detected in the channel for the ROX fluorophore
– The signal of the (IC) DNA amplification product is detected in the channel for the Cy5 fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

– *Candida albicans* DNA is **detected** in a sample if the *Ct* value is determined in the results grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– *Candida glabrata* DNA is **detected** in a sample if the *Ct* value is determined in the results grid in the channel for the JOE fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– *Candida krusei* DNA is **detected** in a sample if the *Ct* value is determined in the results grid in the channel for the ROX fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– *Candida albicans, Candida glabrata и Candida krusei* DNA are **not detected** if the *Ct* value is not determined (absent) in the results grid (the fluorescence curve does not cross the threshold line) in the channels for the FAM, JOE, ROX fluorophores, whereas the *Ct* value determined in the results grid in the channel for the Cy5 fluorophore does not exceed the specified boundary value.
– The result of analysis is **invalid** if the *Ct* value is not determined in the results grid (absent) in the channel for the FAM, JOE, ROX and Cy5 fluorophore. In such cases PCR should be repeated for this sample.

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 2).

Table 2

Results for controls

Control	Stage for control	*Ct* value in channels for fluorophore			
		FAM	**JOE**	**ROX**	**Cy5**
C–	DNA extraction	Absent	Absent	Absent	<boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	<boundary value	<boundary value	<boundary value	<boundary value

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. The *Ct* value determined for the Positive Control of amplification (C+) in the channel for the FAM**,** and/or JOE**,** and/or ROX fluorophore is greater than the specified boundary value or absent. The amplification should be repeated for all the samples in which *Ct* value is absent in the respective channel.
2. The *Ct* value is determined for the Negative Control of Extraction (C–) and/or the Negative Control of Amplification (NCA) in the channel for the FAM**,** and/or JOE**,** and/or ROX fluorophore. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which *Ct* value is determined in the respective channel.

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

11. TRANSPORTATION

AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT** PCR kit are stable until the expiry date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 ºC when not in use.

PCR-mix-1-FL *C.albicans* / *C.glabrata* / *C.krusei* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Transport medium	Nucleic acid extraction kit	Microorganism	Sensitivity, GE/ml [3]
Urogenital swabs	Transport Medium for Swabs **REF** 956-CE, **REF** 987-CE or Transport Medium with Mucolytic Agent **REF** 952-CE, **REF** 953-CE	DNA-sorb-AM	*Candida albicans*	$1x10^3$
			Candida glabrata	$1x10^3$
			Candida krusei	$1x10^3$
Urine	–	DNA-sorb-AM	*Candida albicans*	$2x10^3$
			Candida glabrata	$2x10^3$
			Candida krusei	$2x10^3$

The analytical sensitivity of each microorganism does not change even at high concentrations of two other microorganisms (to 10^9 GE/ml).

13.2. Specificity

The analytical specificity of **AmpliSens® *C.albicans / C.glabrata / C.krusei*-MULTIPRIME-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

Nonspecific responses were absent while testing human DNA samples and DNA samples of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Candida albicans, Candida glabrata, Candida krusei, Neisseria* spp., *Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* types 1 and 2, *CMV,* and *HPV*.

The clinical specificity of **AmpliSens® *C.albicans / C.glabrata / C.krusei*-MULTIPRIME-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines “Real-Time PCR Detection of STIs and Other Reproductive Tract Infections.”, developed by Federal Budget Institute of Science “Central Research

[3] Genome equivalents of microorganism per 1 ml of the sample from transport medium.

Institute for Epidemiology".

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®** ***C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
21.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
26.11.15 LE	Text	Corrections according to the template
	8.1. DNA extraction	Information about controls of extraction was added
	9. Data analysis	The sections was rewritten
	10. Troubleshooting	
	13.1. Sensitivity	The column with the transport media was added
15.12.17 PM	3. Content	The color of a reagent was specified
13.03.18 PM	Footer, 3. Content	**REF** R-F3(iQ)-CE was deleted