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For Professional Use Only

AmpliSens® Florocenosis / *Candida*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® Florocenosis / ***Candida*-FRT** PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection and quantitation of *Candida* genus fungi DNA (*C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis*) in the clinical material (urogenital swabs, oral and oropharyngeal swabs and urine samples) by using polymerase chain reaction with real-time hybridization-fluorescence detection products of amplification.

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

2. PRINCIPLE OF PCR DETECTION

Qualitative detection and quantitation of five types of *Candida* spp. DNA by the multiplex polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection contains three steps: DNA extraction from the clinical material, amplification of the given microorganism DNA and real-time hybridization-fluorescence detection.

Candida spp. detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In the 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® Florocenosis / ***Candida*-FRT** PCR kit is a 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.

The results of amplification of *C.albicans, C.glabrata* and *C.krusei* DNA are registered separately for each species in three different channels. The results of amplification of *C.parapsilosis* and *C.tropicalis* DNA are registered together in the fourth channel. The Internal Control-FL (IC) amplification product is detected in the Cy5.5/Crimson channel.

Detection channel	FAM[1]	JOE[2]	ROX[2]	Cy5[2]	Cy5.5[2]
Identified DNA	DNA *C.albicans*	DNA *C.glabrata*	DNA *C.krusei*	DNA *C.parapsilosis* and *C.tropicalis*	DNA IC

[1] Or the similar detection channel for the detection of the specified fluorophore in accordance with the using instrument.

The quantitation of DNA by real-time PCR is based on the existence of linear dependence between the logarithm of initial DNA-target concentration in the sample and the threshold cycle *Ct*, which corresponds to the start of the exponential growth of the fluorescent signal. The simultaneous amplification with real-time detection of DNA samples and DNA calibrators with the known concentrations is carried out for qualitative analysis. Calibration curve is plotted automatically on the basis of DNA calibrators results. Concentration of corresponding DNA-target is calculated automatically for each sample using the obtained value of threshold cycle and the calibration curve.

3. CONTENT

AmpliSens® Florocenosis / ***Candida*-FRT** PCR kit is produced in 1 form:

AmpliSens® Florocenosis / ***Candida*-FRT** PCR kit variant FRT-100 F, **REF** R-F5-100-FT(RG,CFX)-CE.

AmpliSens® Florocenosis / ***Candida*-FRT PCR kit variant FRT-100 F** includes:

Reagent		*Discription*	*Volume, ml*	*Quantity*
PCR-mix-1-FL Florocenosis / ***Candida***		colorless clear liquid	1,2	1 tube
PCR-mix-2-FRT		colorless clear liquid	0,3	2 tubes
Polymerase (TaqF)		colorless clear liquid	0,03	2 tubes
DNA-buffer		colorless clear liquid	0,5	1 tube
DNA calibrators	**CND1**	colorless clear liquid	0,2	1 tube
	CND2	colorless clear liquid	0,2	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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-12-100-CE protocol).

AmpliSens® Florocenosis / ***Candida*-FRT** PCR kit is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Transport medium for storage and transportation of swabs.

- DNA extraction kit.
- PCR box.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- Disposable pipette tips with filter (up to 100 µl) in racks (for example, Axygen, USA).
- Pipettes (adjustable).
- Disposable powder-free gloves and laboratory coat.
- Tube racks.
- Real-time instruments with five or more separated channels of fluorescence detection (for example, Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
    a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
    b) 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 with the range from 2 to 8 °C.
- Deep-freezer with the range 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 (samples, 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 protective gloves and laboratory cloths, and 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 accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet

in accordance with appropriate biosafety practices.

- Clean and disinfect all samples or reagents 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.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area where 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® Florocenosis / ***Candida*-FRT** PCR kit is used for simultaneous detection and quantitation of *Candida* genus fungi DNA (*C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis*) extracted with DNA extraction kit from the clinical material (urogenital swabs, oral and oropharyngeal swabs, transferred into the Transport Medium with Mucolytic Agent, **REF** 952-CE; **REF** 953-CE, and urine samples).

7. WORKING CONDITIONS

AmpliSens® Florocenosis / ***Candida*-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 kit:

– DNA-sorb-AM, **REF** K1-12-100-CE.

Extract the DNA according to the manufacturer's protocol.

It is forbidden to use EDEM reagents kit or other express methods for DNA extraction.

8.3. Preparing PCR

8.3.1 Preparing tubes for PCR

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

1. Previously prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. The content of one tube with **polymerase (TaqF) (30 µl)** should be transferred into the tube with the **PCR-mix-2–FRT (300 µl)**. Mix carefully avoiding foaming. Mark the tubes by the expiration date.

The prepared mixture intended for 60 reactions. The mixture should be stored at 2–8 °C for 3 months and used when it is necessary.

If the mixture is not to be used in three months, the mixture should be prepared for less number of reactions. For example, mix 150 µl of PCR-mix-2-FRT and 15 µl of polymerase (TaqF) for 30 reactions.

2. Vortex the tube with **PCR-mix-1-FL Florocenosis** / ***Candida*****,** sediment the drops from the tubes cap bu short centrifugation.

   Calculate the reagents volumes for the necessary number of reactions including test and control samples analysis according to the table 1. Take into account that even for one test sample analysis **four control reactions** are to be carried out **CND1, CND2, NCA and C–**.

   The reagents should be taken with reserve. For N samplesanalysis the reagents for (N+1) reactions should be prepared.

Table 1

Scheme of reaction mixture preparation

	Reagent volume for the specified number of reactions, µl	
Reagent volume per one reaction, µl	**10.0**	**5.0**
Number of examining clinical samples	**PCR-mix-1-FL Florocenosis / *Candida*[2]**	**Mixture of PCR-mix-2-FRT and polymerase (TaqF)[2]**
1	**60**	**30**
2	**70**	**35**
3	**80**	**40**
4	**90**	**45**
5	**100**	**50**
6	**110**	**55**
7	**120**	**60**
8	**130**	**65**
9	**140**	**70**
10	**150**	**75**
11	**160**	**80**
12	**170**	**85**
13	**180**	**90**
14	**190**	**95**
15	**200**	**100**
16	**210**	**105**
17	**220**	**110**
18	**230**	**115**
19	**240**	**120**
20	**250**	**125**
21	**260**	**130**
22	**270**	**135**
23	**280**	**140**
24	**290**	**145**
25	**300**	**150**
30	**350**	**175**

3. Prepare the reaction mixture in another tube. The components of the reaction mixture should be mixed directly before the experiment. For one reaction mix:
   - **10 µl** of **PCR-mix-1-FL Florocenosis** / ***Candida***
   - **5 µl** of **PCR-mix-2–FRT** and **polymerase (TaqF)** mixture**.**
4. Take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples.
5. Add **15 µl** of reaction mixture into each tube.
6. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the DNA extraction stage.
7. Carry out the control reactions:

[2] Specified values include one extra reaction and four controls (CND1, CND2, NCA and C–).

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

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

CND1, CND2 - Add **10 µl** of DNA calibrator **CND1** into one tube and **10 µl** of DNA calibrator **CND2** into another tube.

8.3.2. Amplification

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

Table 2

AmpliSens-1 amplification program

Step	***Rotor-type instruments***[3]			***Plate-type instruments***[4]		
	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 Fluorescent signal detection		**60**	30 s Fluorescent signal detection	
	72	15 s		**72**	15 s	

Fluorescent signal is detected in the channels for the FAM, JOE, ROX, Cy5 and Cy5.5 fluorophores.

2. Insert the tubes into the reaction module of the instrument.
3. Run the amplification program with fluorescent signal detection.
4. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

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

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

– The signal of the ***C.glabrata*** DNA amplification product is detected in the channel for the **JOE** fluorophore.

[3] For example, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene (QIAGEN, Germany).
[4] For example, CFX (Bio-Rad, USA).

– The signal of the ***C.krusei*** DNA amplification product is detected in the channel for the **ROX** fluorophore.
– The signal of the ***C.parapsilosis*** and/or ***C.tropicalis*** DNA amplification product is detected in the channel for the **Cy5** fluorophore.
– The signal of the **Internal Control (IC)** DNA amplification product is detected through the channel for the **Cy5.5** fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the level of exponential growth that corresponds the presence (or absence) of a *Ct* value for the certain DNA-target in the corresponding column of the result grid. The calibration curve is plotted and the concentration of detected species of *Candida* spp. is calculated automatically according to the *Ct* values of the DNA calibrators.

The concentrations values of DNA calibrators are specified in the *Important Product Information Bulletin* enclosed in the PCR kit.

The result of analysis is considered reliable only if the results obtained for Negative Control of amplification as well as for the Negative Control of extraction of DNA and DNA calibrator CND1 are correct (see Table 3) and the amplification efficiency coefficient E is in the range specified in the *Important Product Information Bulletin* enclosed in the PCR kit.

Table 3

Results for controls of different stages of the PCR

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM, JOE, ROX, Cy5	Cy5.5
C–	DNA extraction	Absent	<boundary value
NCA	PCR	Absent	Absent
CND1	PCR	<boundary value	Not estimated

The detailed information about the interpretation of the results is represented in the Guidelines enclosed to the PCR kit.

The concentration values of *C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis* DNA reflect the total content of those microorganisms in the clinical material transferred into the transport medium. The initial values of number of copies of *C.albicans, C.glabrata, C.krusei* and *C.parapsilosis+C.tropicalis* DNA in the reaction tube are automatically calculated and given in the corresponding column of the results grid (see Guidelines) on the basis of the set values of DNA calibrators. The obtained values are used for the calculation of number of genome equivalents of the corresponding species of *Candida* in 1 ml of the initial clinical material using the formula:

[Number of copies] *Candida* DNA x K = [Number of genome equivalents] in 1 ml (GE/ml)

The coefficient K for calculations in GE/ml is specified in the *Important Product Information Bulletin* enclosed in the PCR kit.

If the obtained result is greater than $2x10^5$ GE/ml then the result "greater than $2x10^5$ GE/ml" is specified, if the obtained result is less than 200 GE/ml then the result "less than 200 GE/ml" is specified (taking into account the linear range of the kit).

The clinical interpretation of the test results should be carried out by the doctor only on the basis of complex examination of the patient according to the anamnesis data, clinical and epidemiological status, keeping into account the existed clinical and methodological recommendations.

10. TROUBLESHOOTING

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

1. If the *Ct* value is determined for the Negative Control of extraction (C–) and/or Negative Control of amplification (NCA) in the channels for the FAM and/or JOE, and/or ROX, and/or Cy5 fluorophores, the PCR should be repeated for all the samples for which the *Ct* value is defined in the channels for the FAM and/or JOE, and/or ROX, and/or Cy5 fluorophores.
2. If the *Ct* value determined for the DNA calibrators (CND1, CND2) in the channels for the FAM, JOE, ROX, Cy5 fluorophores is greater than the boundary value or absent, or the efficiency coefficient E on the standards curve is less than the value specified in the *Important Product Information Bulletin,* the amplification should be repeated for all of the samples.
3. If the *Ct* values for the test sample in the channels for the FAM, JOE, ROX, Cy5 fluorophores are absent or the obtained number of DNA copies is less than 100 and the *Ct* value in the channel for the Cy5.5 fluorophore is greater than the boundary value or absent, the analysis should be repeated for this sample starting from the stage of DNA extraction.

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

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of the **AmpliSens® Florocenosis** / ***Candida*-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-2-FRT and polymerase (TaqF)). All components of the **AmpliSens® Florocenosis** / ***Candida*-FRT** PCR kit are stable until the expiry date stated on the label.The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-2-FRT and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C.

PCR-mix-1-FL Florocenosis / *Candida* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Transport medium	Nucleic acid extraction kit	Microorganism	PCR kit	Sensitivity, GE/ml[5]
Urogenital swabs, oral and oropharyngeal swabs	Transport Medium for Swabs, REF 956-CE, REF 987-CE or Transport Medium with Mucolytic Agent, REF 952-CE; REF 953-CE or Transport Medium TM-EDEM, REF 1533-CE	DNA-sorb-AM	*C.albicans, C.glabrata, C.krusei, C.parapsilosis, C.tropicalis*	PCR kit variant FRT-100 F	$1x10^2$
Urine[6]	—	DNA-sorb-AM	*C.albicans, C.glabrata, C.krusei, C.parapsilosis, C.tropicalis*	PCR kit variant FRT-100 F	$1x10^2$

13.2. Specificity

The analytical specificity of **AmpliSens® Florocenosis** / ***Candida*-FRT** PCR kit is ensured by the 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.

The nonspecific reactions were absent when testing the human DNA samples and the following microorganisms' DNA: *Candida albicans, C.glabrata, C.krusei, C.parapsilosis, C.tropicalis, Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Chlamydia trachomatis, Neisseria gonorrhoeae, Neisseria* spp.,

[5]Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.
[6] For urine samples the pretreatment for sediment obtaining from 1 ml of urine is required.

Mycoplasma genitalium, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV 1 and 2 types, *CMV, HPV.*

The clinical specificity of **AmpliSens® Florocenosis** / ***Candida*-FRT** PCR kit was confirmed in laboratory clinical trials.

13.3. Linear range

The linear range of measurements for quantitative detection of each detected microorganisms is from 200 to $2x10^5$ GE/ml.

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 to the **AmpliSens® Florocenosis** / ***Candida*-FRT** PCR kit for simultaneous detection and quantitation of *Candida* genus fungi DNA (*C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis*) in the clinical material (urogenital swabs, oral and oropharyngeal swabs and urine samples) by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research 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® Florocenosis** / ***Candida*-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		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	**IC**	Internal control