



IVD
For in Vitro Diagnostic Use

AmpliSens® *Candida albicans*-FRT PCR kit

Instruction Manual

For use with the Rotor-Gene™ 3000 and Rotor-Gene™ 6000 Instruments

iQ5 and iQ iCycler Instruments

RG, iQ

AmpliSens®

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1. INTENDED USE

AmpliSens® *Candida albicans*-FRT PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Candida albicans* DNA in the clinical materials (cervical, urethral scrapes (swabs), urine sediment) by using real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR ASSAY

Candida albicans detection by the polymerase chain reaction (PCR) is based on the amplification of specific region of pathogen genome using specific *Candida albicans* primers. In real-time PCR the amplified product is detected via fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. Monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without having to re-open the reaction tubes after the PCR run. AmpliSens® *Candida albicans*-FRT PCR kit is qualitative test and contains the Internal Control (IC) which must be used in the isolation procedure in order to control the isolation process of each individual specimen and to identify possible reaction inhibition. AmpliSens® *Candida albicans*-FRT PCR kit uses "hot-start", that is guaranteed by separation of nucleotides and Taq-polymerase by wax layer. Melting of wax and mix of reaction components occur only at 95°C, which greatly diminish frequency of nonspecifically primed reactions. In variant FRT-100F "hot-start" is guaranteed by application of polymerase (TaqF). Chemically modified polymerase (TaqF) activates if warmed at 95°C for 15 min.

CONTENTS OF THE KIT

AmpliSens® *Candida albicans*-FRT PCR kit is produced in 3 forms:

AmpliSens® *Candida albicans*-FRT PCR kit variant FRT for use with RG [REF] R-F1 (RG)-CE;

AmpliSens® *Candida albicans*-FRT PCR kit variant FRT for use with iQ [REF] R-F1 (iQ)-CE;

AmpliSens® *Candida albicans*-FRT PCR kit variant FRT-100F [REF] R-F1-F (RG, iQ)-CE.

AmpliSens® *Candida albicans*-FRT PCR kit, variant FRT includes:

| Reagent | Description | Volume (ml) | Amount |
|--|------------------------|-------------|------------------------|
| PCR-mix-1-FEP/FRT <i>Candida albicans</i> (under wax) | colorless, clear fluid | 0.008 | 110 vials of 0.2 ml |
| PCR-mix-2-FL | colorless, clear fluid | 0.77 | 1 vial |
| Positive Control DNA <i>Candida albicans</i> (C+) | colorless, clear fluid | 0.2 | 1 vial |
| DNA-buffer | colorless, clear fluid | 0.5 | 1 vial |
| Negative Control (C-)* | colorless, clear fluid | 1.2 | 1 vial |
| Internal Control-FL** | colorless, clear fluid | 1.0 | 1 vial |

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

** add 10 µl of Internal Control-FL during the DNA isolation procedure directly to the sample/lysis mixture (see "DNA-sorb-AM" [REF] K1-12-100 protocol).

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

AmpliSens® *Candida albicans*-FRT PCR kit, variant FRT-100F includes:

| Reagent | Description | Volume (ml) | Amount |
|---|------------------------|-------------|---------|
| PCR-mix-1-FEP/FRT <i>Candida albicans</i> | colorless, clear fluid | 1.2 | 1 vial |
| PCR-mix-2-FRT | colorless, clear fluid | 0.3 | 2 vials |
| Polymerase (TaqF) | colorless, clear fluid | 0.06 | 1 vial |
| Positive Control DNA <i>Candida albicans</i> (C+) | colorless, clear fluid | 0.2 | 1 vial |
| DNA-buffer | colorless, clear fluid | 0.5 | 1 vial |
| Negative Control (C-)* | colorless, clear fluid | 1.2 | 1 vial |
| Internal Control-FL** | colorless, clear fluid | 1.0 | 1 vial |

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

** add 10 µl of Internal Control-FL during the DNA isolation procedure directly to the sample/lysis mixture (see "DNA-sorb-AM" [REF] K1-12-100 protocol).

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

[REF] R-F1 (RG)-CE; R-F1 (iQ)-CE; R-F1 (RG,iQ)-CE/[VER] 06.11.2009

4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES

- Disposable powder-free gloves
- DNA isolation kit
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters (up to 200 µl)
- Tube racks
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia) Instrument; iQ5 or iQ (Cycler (BioRad, USA) Instrument
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator for 2–8 °C with deep-freezer with temperature no less than –16°C
- Reservoir for disposed tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and put the new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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 specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucose membranes. If these solutions come into contact, rinse 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 techniques of DNA amplification.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.



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

6. SPECIMEN COLLECTION AND HANDLING



In detail, sampling of biological materials for PCR-analysis, transportation and storage is described in handbook of the manufacture [2]. It is recommended to read this handbook before beginning of the work.

AmpliSens® *Candida albicans*-FRT PCR kit is intended to analyze DNA extracted with DNA isolation kits from urogenital tract scrapes (swabs), urine sediment.

7. PROTOCOL

7.1. DNA Isolation

Different manufacturers offer DNA isolation kits. We recommend following nucleic acid extraction kits:

- "DNA-sorb-AM", [REF](#) K1-12-100.



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

7.2. Preparing the PCR

Total reaction volume is 25 µl, the volume of DNA sample is 10 µl.

7.2.1 Preparing tubes for PCR

Variant FRT

1. Prepare the required quantity of the tubes with **PCR-mix-1-FEP/FRT *Candida albicans*** with wax for amplification of DNA study and control samples.
2. Add 7 µl of **PCR-mix-2-FL** to the surface of wax layer of each tube, so that it wouldn't fall under the wax and mix with **PCR-mix-1-FEP/FRT *Candida albicans***.

Variant FRT-100F

1. Prepare required quantity of the tubes for amplification of DNA study and control samples (0.2 ml tubes for 36-Well rotor or 0.1 ml stripes for 72-Well rotor).
2. For performing N reactions (including 2 controls) mix in a new tube: 10*(N+1) µl of **PCR-mix-1-FEP/FRT *Candida albicans***, 5.0*(N+1) µl of **PCR-mix-2-FRT** and 0.5*(N+1) µl of **polymerase (TaqF)**. Vortex the tube, then centrifuge shortly. Transfer 15 µl of prepared mix into each tube.

Steps 3 and 4 are effective for both variants.

3. Using tips with aerosol barrier add 10 µl of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction into prepared tubes.



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

4. Perform 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 <i>Candida albicans</i> to the tube labeled C+ (Positive Control of Amplification). |

7.2.2. Amplification

7.2.2.1. RG

1. Program the Rotor-Gene™ according to manufacturer's manual and Annex 1.1.
2. Create a temperature profile on your Rotor-Gene™ instrument as follows:

AmpliSens-1 RG program

| Step | Temperature, °C | Time | Fluorescence detection | Cycle repeats |
|-----------|-----------------|--------|------------------------|---------------|
| Hold | 95 | 15 min | – | 1 |
| Cycling | 95 | 5 sec | – | 5 |
| | 60 | 20 sec | – | |
| | 72 | 15 sec | – | |
| Cycling 2 | 95 | 5 sec | – | 40 |
| | 60 | 20 sec | FAM/Green, JOE/Yellow | |
| | 72 | 15 sec | – | |



AmpliSens-1 RG general program allows simultaneous conducting of any combination of tests for detection of sexually transmitted diseases pathogens DNA including tests for identifying of *Human Papillomaviruses* by means of AmpliSens HPV HCR PCR kits.



If "multiprime" format tests for detection of sexually transmitted diseases (AmpliSens PCR kits) are carried out simultaneously, the program and template corresponding to those "multiprime" tests should be applied.

3. Fluorescence detection in fluorometer channel FAM/Green and JOE/Yellow is on the 2-nd pass (60°C).
4. Make the adjustment of the fluorescence channel sensitivity according to Annex 1.1.

7.2.2.2. iQ

1. Program the iQ™ according to manufacturer's manual and Annex 2.1.
2. Create a temperature profile on your iQ™ instrument as follows:

AmpliSens-1 iQ program

| Step | Temperature, °C | Time | Fluorescence detection | Cycle repeats |
|-----------|-----------------|--------|------------------------|---------------|
| Hold | 95 | 15 min | – | 1 |
| Cycling | 95 | 5 sec | – | 5 |
| | 60 | 20 sec | – | |
| | 72 | 15 sec | – | |
| Cycling 2 | 95 | 5 sec | – | 40 |
| | 60 | 30 sec | FAM, HEX | |
| | 72 | 15 sec | – | |

- Fluorescence detection in fluorometer channel FAM and HEX is on the 2-nd pass (60°C).
- Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.

8. DATA ANALYSIS

RG. Internal Control is detected in the JOE /Yellow fluorescence channel, *Candida albicans* DNA is detected in the FAM /Green fluorescence channel.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

iQ. Internal Control is detected in the HEX fluorescence channel, *Candida albicans* DNA is detected in the FAM fluorescence channel.

See **Appendix 2** for data analysis settings for iQ5 or iQiCycler.

Results interpretation

The results are interpreted by the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 or iQ5 or iQiCycler Instrument by the crossing (or not) of the fluorescence curve with the threshold line.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

Results for controls

| Control | Stage for control | Ct in channel | | Interpretation |
|---------|-------------------|---------------|------------|----------------|
| | | FAM /Green | JOE/Yellow | |
| C- | DNA isolation | Neg | Pos (< X*) | OK |
| NCA | Amplification | Neg | Neg | OK |
| C+ | Amplification | Pos (< Z*) | Neg | OK |

*For X, Z values see Appendix 1 in case of using Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument or Appendix 2 in case of using iQ5 or iQiCycler Instrument.

- The sample is considered to be positive for *Candida albicans* if its Ct value is defined in the results grid in FAM/Green channel.
- The sample is considered to be negative for *Candida albicans* if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM/Green channel and in the results grid in the JOE/Yellow / HEX channel the Ct value doesn't exceed X.

9. TROUBLESHOOTING

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

- If Ct value is absent on both JOE/Yellow/HEX and FAM/Green channels or the Ct value in JOE/Yellow / HEX channel is higher than X, PCR reaction should be repeated. If the same result is achieved, the sample extraction process should be repeated. If the IC signal of this sample was detected normally during any other PCR test, then the second examination of the extraction stage should be omitted (in case of using iQ5 or iQiCycler Instrument).
- If the Ct value is present for the Negative Control of extraction (C-) on FAM/Green channel and/or for Negative Control of Amplification on FAM, JOE/Yellow/HEX channels in the results grid, it indicates the contamination of reagent or samples. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures to detect and eliminate the source of contamination are to be taken.
- If no signal is detected for Positive Controls of amplification, it can suggest incorrect programming of the temperature profile of used Instrument, incorrect configuration of the PCR reaction or storage conditions for kit components has not complied with manufacturer instruction, or the reagents kit has expired. Programming of the used Instrument, storage conditions, and the expiration date of the reagents should be checked, and then the PCR should be repeated
- If a positive result (fluorescence curve crosses the threshold line) is registered for the sample that has a fluorescence curve without a typical exponential growth (the graph is linear). This can suggest incorrect setting of the threshold line or incorrect calculation of base line parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, the PCR should be

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repeated for the sample (in case of using iQ5 or iQiCycler Instrument).

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-FRT** PCR kit (except for Polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at temperature between 2 °C and 8 °C when not in use. All components of the **AmpliSens® Candida albicans-FRT** PCR kit are to be stable until labeled expiration date.



Polymerase (TaqF) and PCR-mix-2-FRT should be stored at minus 16°C.

11. SPECIFICATIONS

11.1. Sensitivity

Analytical Sensitivity of **AmpliSens Candida albicans-FRT** PCR kit is no less than 1x10⁴ genome equivalents per 1 ml of sample (GE/ml).



Claimed analytical features of **AmpliSens® Candida albicans-FRT** PCR kit are guaranteed only when additional reagents kit, "DNA-sorb-AM", is used.

11.2. Specificity

Specificity of **AmpliSens® Candida albicans-FRT** 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-FRT** PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES

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

14. EXPLANATION OF SYMBOLS



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQiCycler



Negative control

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