# AmpliSens® *Cryptococcus neoformans*-FRT PCR kit

CE IVD

For Professional Use Only

# Instruction Manual

## KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number | ⚠ | Caution |
| LOT | Batch code | Σ | Contains sufficient for <n> tests |
| IVD | *In vitro* diagnostic medical device | | Use-by Date |
| VER | Version | | Consult instructions for use |
| | Temperature limit | | Keep away from sunlight |
| | Manufacturer | NCA | Negative control of amplification |
| | Date of manufacture | C– | Negative control of extraction |
| EC REP | Authorized representative in the European Community | C+ | Positive control of amplification |
| | | IC | Internal control |

## 1. INTENDED USE

**AmpliSens® *Cryptococcus neoformans*-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Cryptococcus neoformans* DNA in the biological material (cerebrospinal fluid, bronchoalveolar lavage, sputum, blood, skin lesions aspirate, viscera biopsy and autopsy material) by using real-time hybridization-fluorescence detection of amplified products.

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

## 2. PRINCIPLE OF PCR DETECTION

*Cryptococcus neoformans* detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Cryptococcus neoformans* 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® *Cryptococcus neoformans*-FRT** PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87 (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® *Cryptococcus neoformans*-FRT** PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and dUTP. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

The results of amplification are registered in the following fluorescence channels:

Table 1

| Channel for fluorophore | FAM | JOE |
|---|---|---|
| DNA-target | Internal Control STI-87L (IC) DNA | *Cryptococcus neoformans* DNA |
| Target gene | Artificially synthesized sequence | ITS-2 gene DNA |

## 3. CONTENT

**AmpliSens® *Cryptococcus neoformans*-FRT** PCR kit is produced in 1 form:

**variant FRT-100 F**, REF R-F4-F(RG,iQ)-CE

**Variant FRT-100 F** includes:

| Reagent | Description | Volume, ml | Quantity |
|---|---|---|---|
| **PCR-mix-1-FRT *Cryptococcus*** | clear liquid from colorless to light lilac colour | 1.2 | 1 tube |
| **PCR-mix-2-FRT** | colorless clear liquid | 0.6 | 1 tube |
| **Polymerase (TaqF)** | colorless clear liquid | 0.06 | 1 tube |
| **DNA-buffer** | colorless clear liquid | 0.5 | 1 tube |
| **Positive control DNA *Crypt.*-1 ($C+_1$)** | colorless clear liquid | 0.2 | 1 tube |
| **Positive control DNA *Crypt.*-2 ($C+_2$)** | colorless clear liquid | 0.2 | 1 tube |
| **Negative control (C–)*** | colorless clear liquid | 1.2 | 2 tubes |
| **Internal control STI-87 (IC)**** | colorless clear liquid | 0.6 | 2 tubes |

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

** add **10 µl** of **Internal control STI-87 (IC)** during the DNA extraction procedure directly to the sample/lysis mixture (see **RIBO-prep**, REF K2-9-Et-100-CE protocol).

**Variant FRT-100 F** is intended for 110 reactions (including controls).

## 4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free 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); iCycler iQ5 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  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 (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 protective gloves and laboratory cloths, and 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance 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 inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- 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, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in 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

**NOTE** 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® *Cryptococcus neoformans*-FRT** PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (cerebrospinal fluid, bronchoalveolar lavage, sputum, blood, skin lesions aspirate, viscera biopsy and autopsy material).

## 7. WORKING CONDITIONS

**AmpliSens® *Cryptococcus neoformans*-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:

– **RIBO-prep**, REF K2-9-Et-100-CE.

**NOTE:** Extract the DNA according to the manufacturer's protocol.

### 8.2. Preparing PCR

#### 8.2.1 Preparing tubes for RT-PCR

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

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF).** For this, add the whole volume of **polymerase (TaqF) (60 µl)** into the tube with **PCR-mix-2-FRT (600 µl).** Carefully vortex the tube, avoiding foaming. Centrifuge briefly (1-2 s) to sediment the drops. Mark the preparation date on the tube.
   Prepared mixture is intended for analysis of 120 samples. Store the mixture at the temperature from 2 to 8 °C for 3 months and use as it is necessary.

**NOTE:** If the prepared mixture cannot be used within 3 months prepare the mixture for less number of reactions. For example, mix **150 µl of PCR-mix-2-FRT** and **15 µl of polymerase (TaqF)** (prepared mixture is intended for 30 reactions).

2. Prepare the reaction mixture. Take into account that it is necessary to carry out 3 control reactions (positive controls of amplification - Positive control DNA *Crypt.*-1 ($C+_1$), Positive control DNA *Crypt.*-2 ($C+_2$), negative control of amplification - DNA-buffer) even for 1 test sample. Moreover take the reagents with a reserve: prepare the reagents for (N+1) reactions for analysis of N samples.
3. Mix in a new tube **PCR-mix-1-FRT *Cryptococcus*** and prepared mixture of **PCR-mix-2-FRT** and **polymerase (TaqF).** Calculate the reagents volumes on the basis that for 1 reaction it is needed:
   **10 µl of PCR-mix-1-FRT *Cryptococcus*,**
   **5 µl of mixture of PCR-mix-2-FRT and polymerase (TaqF).**

One can calculate the reagents volumes for needed number of reactions including test and control samples analysis in accordance with the scheme of reaction mixture preparation (see Table 2).

Table 2

**Scheme of reaction mixture preparation**

| | Reagent volumes for specified number of reactions | |
|---|---|---|
| **Reagent volume per one reaction, µl** | **10.0** | **5.0** |
| **Number of clinical samples** | **PCR-mix-1-FRT *Cryptococcus*[1]** | **Mixture of PCR-mix-2-FRT и polymerase (TaqF)[1]** |
| **1** | **50** | **25** |
| **2** | **60** | **30** |
| **3** | **70** | **35** |
| **4** | **80** | **40** |
| **5** | **90** | **45** |
| **6** | **100** | **50** |
| **7** | **110** | **55** |
| **8** | **120** | **60** |
| **9** | **130** | **65** |
| **10** | **140** | **70** |
| **11** | **150** | **75** |
| **12** | **160** | **80** |
| **13** | **170** | **85** |
| **14** | **180** | **90** |
| **15** | **190** | **95** |
| **16** | **200** | **100** |
| **17** | **210** | **105** |
| **18** | **220** | **110** |
| **19** | **230** | **115** |
| **20** | **240** | **120** |
| **21** | **250** | **125** |
| **22** | **260** | **130** |
| **23** | **270** | **135** |
| **24** | **280** | **140** |
| **25** | **290** | **145** |
| **30** | **340** | **170** |

4. Take the required number of tubes for amplification of the DNA obtained from clinical and control samples.
5. Add **15 µl** of prepared reaction mixture to each tube.
6. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the DNA extraction stage from test and control samples to the tubes with reaction mixture.
7. Carry out the control amplification reactions:

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

**$C+_1$, $C+_2$** — Add **10 µl** of **Positive control DNA *Crypt.*-1 ($C+_1$)** to the tube labeled $C+_1$, add **10 µl** of **Positive control DNA *Crypt.*-2 ($C+_2$)** to another one tube labeled $C+_2$

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

#### 8.2.2. Amplification

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

Table 3

**AmpliSens-1 amplification program**

| | Rotor-type instruments[2] | | | Plate-type instruments[3] | | |
|---|---|---|---|---|---|---|
| **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 | | **60** | 30 s | |
| | **72** | 15 s | | **72** | 15 s | |

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores (when another tests are performed simultaneously the detection in another channels is enabled).

2. Adjust the fluorescence channel sensitivity according to the *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 two channels:

– The signal of the IC DNA (Internal control STI-87 (IC)) amplification product is detected in the channel for the FAM fluorophore.
– The signal of the *Cryptococcus neoformans* DNA amplification product is detected in the channel for the JOE 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 cDNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

– ***Cryptococcus neoformans*** DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore is not more than the boundary *Ct* value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– ***Cryptococcus neoformans*** DNA is **not detected** if the *Ct* value is not determined (absent) or greater than the boundary *Ct* value specified in the *Important Product Information Bulletin* in the channel for JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is not more than the boundary *Ct* value specified in the *Important Product Information Bulletin*.
– The result is **invalid** if the *Ct* value is not determined (absent) in the channel for FAM fluorophores, whereas the *Ct* value in the channel for the JOE fluorophore is not determined (absent) or greater than the specified boundary *Ct* value. In such cases, the PCR analysis of this clinical sample should be repeated.

**NOTE:** 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 Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 4).**

Table 4

**Results for controls**

| **Control** | **Stage for control** | ***Ct* value in the channel for fluorophore** | |
|---|---|---|---|
| | | **FAM** | **JOE** |
| C– | DNA extraction | **<**boundary value | Absent |
| NCA | PCR | Absent | Absent |
| $C+_1$ | PCR | **<**boundary value | **<**boundary value |
| $C+_2$ | PCR | **<**boundary value | **<**boundary value |

## 10. TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Controls of Amplification ($C+_1$ and $C+_2$) in the channel for the **JOE** fluorophore is greater than the boundary *Ct* value or absent, the amplification should be repeated for all samples in which *Cryptococcus neoformans* DNA was not detected.
2. If the *Ct* value is determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channel for the **JOE** fluorophore, the PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which *Cryptococcus neoformans* DNA was detected. Take measures to detect the source of contamination.

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

## 11. TRANSPORTATION

**AmpliSens® *Cryptococcus neoformans*-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® *Cryptococcus neoformans*-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FRT *Cryptococcus*, PCR-mix-2-FRT, and polymerase (TaqF)). All components of the **AmpliSens® *Cryptococcus neoformans*-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.

**NOTE:** PCR-mix-1-FRT *Cryptococcus*, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C.

**NOTE:** PCR-mix-1-FRT *Cryptococcus* is to be kept away from light.

[1] The volumes are specified with account of reserve (one extra reaction) and necessity of carrying out 3 controls of amplification (positive controls - Positive control DNA *Crypt.*-1 ($C+_1$), Positive control DNA *Crypt.*-2 ($C+_2$) and negative control - DNA-buffer).

[2] For example, Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).

[3] For example, iCycler iQ5 (Bio-Rad, USA).

## 13. SPECIFICATIONS

### 13.1. Analytical sensitivity

| Biological material | Nucleic acid extraction kit | PCR kit | Sensitivity, copies/ml |
|---|---|---|---|
| - cerebrospinal fluid,<br>- bronchoalveolar lavage,<br>- sputum,<br>- blood,<br>- skin lesions aspirate,<br>- viscera biopsy and autopsy material | RIBO-prep | variant FRT-100 F | 400 |

### 13.2. Analytical specificity

The analytical specificity of **AmpliSens® *Cryptococcus neoformans*-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.

Specificity of PCR kit for qualitative detection of *Cryptococcus neoformans* was studied on strains of fungi: *Penicillium brevicompactum, Penicillium chrysogenum, Trichoderma harzianum, Trichothecium roseum, Trichoderma viride, Trichoderma koningii, Fusarium solani, Fusarium poae, Fusarium oxysporum, Fusarium sambucinum, Fusarium verticillioides, Mucor plumbeus, Mucor hiemalis, Mucor racemosus, Mucor pusillus, Aspergillus versicolor, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Rhizopus stolonifer, Rhizopus oryzae, Rhizopus microsporus, Scedosporium apiospermum, Trichosporon beigelii, Neurospora sitophila, Stachybotrys chartarum, Paecilomyces fulvus, Cladosporium cladosporioides, Wallemia sebi, Geotrichum candium, Candida albicans, Candida glabrata, Candida krusei; and* human DNA. Nonspecific reactions (falce-positive results) were absent.

The clinical specificity of **AmpliSens® *Cryptococcus neoformans*-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.
2. Guidelines to the **AmpliSens® *Cryptococcus neoformans*-FRT** PCR kit for qualitative detection of *Cryptococcus neoformans* DNA in the biological material (cerebrospinal fluid, bronchoalveolar lavage, sputum, blood, skin lesions aspirate, viscera biopsy and autopsy material) 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 the **AmpliSens® *Cryptococcus neoformans*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

**List of Changes Made in the Instruction Manual**

| VER | Location of changes | Essence of changes |
|---|---|---|
| 10.01.19 EM | 2. Principle of PCR detection | The information about the enzyme UDG was added. The information about «hot-start» was corrected |
| 13.03.19 DV | 3. Content | The color of the reagent was specified |
| 13.07.20 MM | Through the text | The text formatting was changed |
| | 2. Principle of PCR detection | The table with targets was added |
| | Footer | The phrase “Not for use in the Russian Federation” was added |
| 18.03.21 VA | — | The name, address and contact information for Authorized representative in the European Community was changed |

**AmpliSens®**


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