# AmpliSens® Florocenosis / *Mycoplasma*-FRT PCR kit

IVD

For Professional Use Only

# Instruction Manual

## KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number | | Caution |
| LOT | Batch code | | Contains sufficient for <n> tests |
| IVD | *In vitro* diagnostic medical device | | Use-by Date |
| VER | Version | | Consult instructions for use |
| | Temperature limit | NCA | Negative control of amplification |
| | Manufacturer | C– | Negative control of extraction |
| | Date of manufacture | C+ | Positive control of Amplification |
| EC REP | Authorized representative in the European Community | IC | Internal control |
| FBIS CRIE | Federal Budget Institute of Science "Central Research Institute for Epidemiology | | |

## 1. INTENDED USE

**AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection and quantification of *Ureaplasma parvum, Ureaplasma urealyticum* and *Mycoplasma hominis* DNA in the clinical material (urogenital swabs from cervical canal, vagina, urethra and first pass of urine) using real-time hybridization-fluorescence detection.

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

## 2. PRINCIPLE OF PCR DETECTION

*Ureaplasma parvum, Ureaplasma urealyticum* and *Mycoplasma hominis* DNA detection is based on the amplification of the pathogen genome specific region using specific primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that 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.

Clinical material is taken and placed in a transport medium for storage and transportation of clinical samples. DNA is extracted from the clinical samples and used for PCR with hybridization fluorescent detection (real-time PCR). Detection and quantification of *Ureaplasma parvum* DNA, *Ureaplasma urealyticum* DNA, and *Mycoplasma hominis* DNA is performed by using multiplex real-time PCR.

Quantitative detection of DNA by real-time PCR is based on the linear dependence between initial concentration of target DNA in a test sample and a cycle when the fluorescent signal begins to increase exponentially (cycle threshold (*Ct*)). For quantitative detection, DNA of clinical samples is amplified simultaneously with DNA calibrators (samples with known concentration of DNA-target). The results of amplification of DNA calibrators are used for construction of a calibration curve and calculation of the target DNA concentration in test samples.

In the **AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit the concentration of *U.parvum, U.urealyticum,* and *M.hominis* DNA can be determined in two variants. In the first variant the number of genome equivalents of microorganism cells per 1 ml of clinical sample (GE/ml) is determined. Thus obtained values reflect absolute concentration of these microorganisms in the clinical material. In the second variant the ratio between *U.parvum, U.urealyticum, M.hominis* genomes and genomes of human mucosa cells is calculated. In this case, PCR-mix contains not only primers and probes for *U.parvum, U.urealyticum,* and *M.hominis* DNA but also the primers and probes for human β-globin gene fragment. DNA calibrator solutions contain *U.parvum, U.urealyticum,* and *M.hominis* DNA calibrators as well as human DNA calibrators. Thus obtained relative values of *U.parvum, U.urealyticum,* and *M.hominis* DNA concentration reflect the density of the microorganisms on the mucosa. In addition, human DNA serves as endogenous internal control which helps to monitor the quality of clinical material sampling.

**AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit uses "hot-start," which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using a 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 | ROX | Cy5 |
|---|---|---|---|---|
| DNA-target | *U.parvum* | *U.urealyticum* | *M.hominis* | Internal Control-FL (IC) DNA |
| Target gene | Urease gene | Urease gene | gene 16S rRNA | β-globin gene |

## 3. CONTENT

**AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit is produced in 1 form:
**variant FRT-100 F**, REF R-B75-100-FT(RG,iQ,Mx)-CE.

**Variant FRT-100 F** includes:

| Reagent | | Description | Volume, ml | Quantity |
|---|---|---|---|---|
| **PCR-mix-1-FL *U.parvum* / *U.urealyticum* / *M.hominis*** | | 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 |
| **DNA-buffer** | | colorless clear liquid | 0.5 | 1 tube |
| **DNA calibrators** | **UG1** | colorless clear liquid | 0.1 | 1 tube |
| | **UG2** | colorless clear liquid | 0.1 | 1 tube |
| **Negative control (C–)*** | | colorless clear liquid | 1.2 | 1 tube |

* must be used in the extraction procedure as Negative Control of Extraction.
**Variant FRT-100 F** 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 barriers (up to 100 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with 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 iQ or iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stragagene, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml).
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed 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 at the temperature 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 clinical materials for PCR-analysis, transportation and storage is described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

**AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from urogenital swabs (placed in the transport media recommended or manufactured by FBIS CRIE) and first pass of urine samples

## 7. WORKING CONDITIONS

**AmpliSens® Florocenosis / *Mycoplasma*-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**.

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

**NOTE:** DNA extraction using EDEM reagent kit or any express methods is unacceptable.

### 8.2. Preparing PCR

#### 8.2.1 Preparing tubes for PCR

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

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.

Reaction mixture components should be mixed just before analysis. Mix reagents for one reaction in the following proportion:

– **10 µl of PCR-mix-1-FL *U.parvum* / *U.urealyticum* / *M.hominis***

– **5 µl of PCR-mix-2-FRT and polymerase (TaqF)** mixture**.**

1. Before starting work, it is necessary to prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. Transfer the entire content of one tube with **polymerase (TaqF) (30 µl)** to the tube with **PCR-mix-2-FRT (300 µl)**. Mark each tube with the mixture preparation date..

**NOTE:** The prepared mixture is intended for analysis of 60 samples. The mixture should be stored at 2–8 °C for not longer than 3 months and used as needed.

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

2. Vortex the tube with **PCR-mix-1-FL *U.parvum* / *U.urealyticum* / *M.hominis***. Centrifuge shortly to remove the drops from the caps of the tubes.
   Calculate the required number of reactions including the test and control samples according to Table 2. Note that even for analysis of one test DNA sample, it is necessary to run **4 controls**: **DNA-calibrators (UG1 and UG2), Negative Control of Extraction (C–) and the Negative Control of Amplification (NCA)**.
   It is necessary to take reagents for one extra reaction: for N tests, prepare reagents for (N+1) reactions.

Table 2

**Scheme of reaction mixture preparation**

| | Reagent volume for the specified number of reactions, µl | |
|---|---|---|
| **Reagent volume for one reaction, µl** | **10 µl** | **5 µl** |
| **Number of clinical samples to be tested** | **PCR-mix-1-FL *U.parvum* / *U.urealyticum* / *M.hominis*** | **Mixture of PCR-mix-2-FRT and polymerase (TaqF)** |
| **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** |

**NOTE:** Values are given with account of one extra reaction and four controls: UG1, UG2, C–, and NCA.

3. Prepare the reaction mixture in an individual tube. Mix **PCR-mix-1-FL *U.parvum* / *U.urealyticum* / *M.hominis* with the mixture of PCR-mix-2-FRT and polymerase (TaqF)** prepared as described in point 1 of Section 8.2.1.
4. Take the required number of tubes for amplification of DNA from clinical and control samples.
5. Transfer **15 µl** of prepared reaction mixture to the tubes.
6. Add **15 µl** of reaction mixture into each tube.
7. Carry out the control reactions:

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

**DNA calibrator UG1** — Add **10 µl** of **DNA calibrator UG1** to the tube labeled UG1.

**DNA calibrator UG2** — Add **10 µl** of **DNA calibrator UG2** to the tube labeled UG2.

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

### 8.2.2. Amplification

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

Table 3

**AmpliSens-1 amplification program**

| Step | Rotor-type instruments[1] | | | Plate-type instruments[2] | | |
|---|---|---|---|---|---|---|
| | **Temperature, °C** | **Time** | **Cycles** | **Temperature, °C** | **Time** | **Cycles** |
| Hold | **95** | 15 min | 1 | **95** | 15 min | 1 |
| Cycling 1 | **95** | 5 s | 5 | **95** | 5 s | 5 |
| | **60** | 20 s | | **60** | 20 s | |
| | **72** | 15 s | | **72** | 15 s | |
| Cycling 2 | **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 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 the *Important Product Information Bulletin*.
3. Insert tubes into the reaction module of the device.
4. 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 ***U.parvum* DNA** amplification product is detected in the channel for the FAM fluorophore,

– The signal of the ***U.urealyticum* DNA** amplification product is detected in the channel for the JOE fluorophore,

– The signal of the ***M. hominis* DNA** amplification product is detected in the channel for the ROX fluorophore,.

– The signal of the **Internal Control DNA (β-globin)** 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 *Ct* value of the DNA sample in the corresponding column of the result grid. Based on the *Ct* values of DNA-calibrators a calibration line is plotted and it is used for the estimation of concentration of *U.parvum, U.urealyticum, M.hominis* and human DNA.

**NOTE:** The concentrations values of DNA calibrators are specified in the *Important Product Information Bulletin*.

The final concentration of *U.parvum, U.urealyticum,* and *M.hominis* DNA can be expressed as absolute and relative (normalized) values

**Absolute concentration of *U.parvum, U.urealyticum,* and *M.hominis***

The absolute concentration of *U.parvum, U.urealyticum* и *M.hominis* shows indicates the total content of these microorganisms in the clinical material placed in transport media. According to calibrator values, the instrument software automatically calculates initial concentrations (DNA copies/reaction) of *U.parvum, U.urealyticum* and *M.hominis* and displays them in the result grid. The obtained values are used for calculation of the number of genome equivalent in 1 ml of clinical sample:

**[Number of copies] DNA *Up (Uu, Mh)* X 200 = [Number of GE] *Up (Uu, Mh)* in 1 ml (GE/ml)**

**Relative (normalized) concentration of *U.parvum, U.urealyticum,* and *M.hominis***

The normalized concentration values indicate number of microorganism cells relatively to the number of human cells. In addition, concentration of human DNA reflects material sampling quality. On the basis of the specified values of calibrators of *U.parvum, U.urealyticum, M.hominis* and human DNA the instrument software automatically calculates the initial number of *U.parvum, U.urealyticum* and *M.hominis* DNA copies as well as the number of human DNA copies in a reaction and displays it in the result table. The obtained *U.parvum, U.urealyticum* and *M.hominis* DNA genome equivalents are normalized for 100,000 human cells by the following formula:

$$\frac{\text{[Number of copies] } DNA\ Up\ (Uu, Mh)}{\text{[Number of copies] human DNA}} \times 200{,}000 = GE\ Up\ (Uu, Mh)\ \text{per } 10^5 \text{ human cells}$$

**NOTE:** For details, see the Guidelines [2].

## 10. TROUBLESHOOTING

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

1. If the signal is detected for the Negative Control of Extraction (C–) and/or for Negative Control of Amplification (NCA) in the channels for the FAM, JOE and ROX fluorophores, the PCR-analysis should be repeated for all samples with *Ct* values determined in the channels for the FAM, JOE and ROX fluorophores.
2. If ***Calc Conc*** value is greater than 5 copies/reaction (500 copies/ml) appears in the results grid for the Negative Control of Extraction (C–) and/or Negative Control of Amplification (NCA) in the channel for the Cy5 fluorophore, it indicates contamination of reagents or samples. In such cases the results of analysis are considered invalid. The PCR-analysis analysis must be repeated (beginning with DNA extraction stage) for those samples that have a signal in the channels for the FAM, JOE and ROX fluorophores and measures to detect and eliminate the source of contamination must be taken.
3. If the *Ct* value for DNA calibrators (UG1 and UG2) is not determined (absent) in the channels for the FAM, JOE, ROX and Cy5fluorophores or if the difference between *Ct* values does not fall in the range specified in the *Important Product Information Bulletin*, the PCR should be repeated for all samples.
4. If human DNA is absent in the clinical material, the material sampling and PCR analysis should be repeated

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

## 11. TRANSPORTATION

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

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

## 12. STABILITY AND STORAGE

All components of the **AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit (except for PCR-mix-2-FRT and polymerase (TaqF)) are to be stored at 2–8 °C when not in use.
All components of the **AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit are stable until the expiry date on the label. PCR kit **variant FRT-100 F** can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, PCR kit **variant FRT-100 F** should be unpacked in accordance with the storage temperatures for each component. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

**NOTE:** 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-FL *U.parvum* / *U.urealyticum* / *M.hominis* is to be kept away from light.

## 13. SPECIFICATIONS

### 13.1. Analytical sensitivity

The analytical sensitivity of **AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit is the following:

| Clinical material | Transport medium | Nucleic acid extraction kit | Microorganism | Analytical sensitivity[3], GE/ml |
|---|---|---|---|---|
| Urogenital swabs | Transport medium with mucolytic agent or Transport medium TM-EDEM | DNA-sorb-AM | *Ureaplasma parvum* | $1x10^3$ |
| | | | *Ureaplasma urealyticum* | $1x10^3$ |
| | | | *Mycoplasma hominis* | $1x10^3$ |
| Urine (first pass) | — | DNA-sorb-AM | *Ureaplasma parvum* | $2x10^3$ |
| | | | *Ureaplasma urealyticum* | $2x10^3$ |
| | | | *Mycoplasma hominis* | $2x10^3$ |

The linear measurement range for quantitative detection of these microorganisms is $10^3$-$10^7$ GE/ml.

### 13.2. Analytical specificity

The analytical specificity of **AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit is ensured by selection of primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis.
Nonspecific reactions were absent while testing DNA samples of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Chlamydia trachomatis, Neisseria gonorrhoeae, Neisseria* spp., *Mycoplasma genitalium, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* type 1 and 2, *CMV, HPV*.
The clinical specificity of **AmpliSens® Florocenosis / *Mycoplasma*-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 PCR kits for simultaneous qualitative detection and quantitation of STIs in the clinical 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 **AmpliSens® Florocenosis / *Mycoplasma*-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 |
|---|---|---|
| 05.11.15 PM | Through the text | Corrections according the template |
| 15.12.17 PM | 3. Content | The colour of PCR-mix-1-FL *U.parvum* / *U.urealyticum* / *M.hominis* was specified |
| 10.01.19 EM | 2. Principle of PCR detection | The information about the enzyme UDG was added. The information about «hot-start» was corrected |
| 17.04.20 KK | Through the text | The text formatting was changed |
| | Footer | The phrase “Not for use in the Russian Federation” was added |
| | 2. Principle of PCR detection | The table with targets was added |
| 16.03.21 EM | — | The name, address and contact information for Authorized representative in the European Community was changed |
| 30.11.21 MM | 12. Stability and storage | The information about storage conditions for 3 months from the date of manufacture and subsequent unpacking was added |
| | Through the text | The reference numbers of nucleic acid extraction kits and transport mediums were deleted |

**AmpliSens®**


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[3] Quantity of genome equivalents (GE) of microorganisms in 1 ml of clinical material placed in the transport medium specified.