CE

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For Professional Use Only

AmpliSens® Florocenosis / Mycoplasma-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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* DNA*, Ureaplasma urealyticum* DNA, and *Mycoplasma hominis* DNA in clinical materials (urogenital swabs, urine) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Collected clinical material is placed in a transport medium and used for DNA extraction. Detection and quantification of *Ureaplasma parvum* DNA*, Ureaplasma urealyticum* DNA, and *Mycoplasma hominis* DNA is performed by using multiplex real-time PCR. Quantitative determination of DNA by real-time PCR is based on linear correlation between initial DNA concentration in a sample and the first cycle of exponential growth of fluorescence (Cycle threshold (*Ct*)). Cycle threshold *(Ct)* is a cycle when fluorescence curve crosses the threshold line. DNA-amplification of clinical samples is performed simultaneously with DNA-calibrators (samples with known concentration of DNA-target). Amplification of DNA-calibrators enables to plot a calibration line which is used for determination of DNA concentration in clinical samples.

AmpliSens® Florocenosis / Mycoplasma-FRT PCR kit allows determination of DNA concentration of *U.parvum, U.urealyticum,* and *M.hominis* in two variants. In the first variant concentration is determined as quantity of genome equivalents of microorganisms in 1 ml of clinical material (GE/ml). Therefore, calculated values reflect absolute concentration of these microorganisms in clinical material. In the second variant the ratio of GE of *U.parvum, U.urealyticum, M.hominis* to GE of human cells is calculated. Therefore, PCR-mix contains primers and probes not only to *U.parvum, U.urealyticum,* and *M.hominis* but also to human β-globin fragment. In DNA-calibrators human DNA is present along with *U.parvum, U.urealyticum,* and *M.hominis* DNA. Calculated values of *U.parvum, U.urealyticum,* and *M.hominis* DNA concentration show microbial contamination of mucous cells. In addition, human DNA serves as endogenous internal control for assessment of the adequacy of sampling and storage of clinical material.

3. CONTENT

AmpliSens® Florocenosis / Mycoplasma-FRT PCR kit is produced in 2 forms:

AmpliSens® Florocenosis / Mycoplasma-FRT PCR kit variant FRT-100 F (for use with RG,

iQ, Mx) **REF** R-B75-100-FT(RG,iQ,Mx)-CE.

AmpliSens® Florocenosis / Mycoplasma-FRT PCR kit variant FRT-100 F in bulk[1] (for use with RG, iQ, Mx), **REF** R-B75-100-FT(RG,iQ,Mx)-CE-B.

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

Reagent		***Description***	***Volume, ml***	***Quantity***
PCR-mix-1-FL *U.parvum* / *U.urealyticum* / *M.hominis*		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	**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.

AmpliSens® Florocenosis / Mycoplasma-FRT PCR kit is intended for 110 reactions (including controls and calibrators).

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 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia, Rotor-Gene Q (QIAGEN, Germany), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stragagene, USA)).
- Disposable polypropylene tubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium hypochlorite or another suitable disinfectant.
- Avoid contact with the skin, eyes, and mucosa. If skin, eyes, or mucosa contact, immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- The laboratory process 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 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 from urogenital swabs and urine sediment (use the first portion of the morning specimen) placed in the transport media recommended or manufactured by CRIE.

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 , **REF** K1-12-100-CE,
- Additional reagent, Transport Medium with Mucolytic Agent **REF** 952-CE or Transport Medium TM-EDEM **REF** 1533-CE is required.

Extract DNA according to the manufacturer's instructions.

Extraction of DNA by express methods (for example, EDEM reagent kit, manufactured by CRIE) is not recommended.

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.**

1. Prepare the reaction mixture straight before the test. Reagents should be mixed in the following proportion (given volumes are calculated for one reaction):
   - **10 µl of PCR-mix-1-FL *U.parvum / U.urealyticum / M.hominis***
   - **5 µl of mixture of PCR-mix-2-FRT and polymerase (TaqF).**
2. 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)**. Avoid foaming while vortexing the tube. Indicate the mixture preparation date on the tube.

The prepared mixture is intended for analysis of 60 samples. The mixture should be stored at 2–8 °C for up to 3 months and used as required.

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.

3. 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 Appendix 1. Note that even for analysis of one DNA test sample in the quantitative format, it is necessary to run **4 controls**: **DNA-calibrators (UG1 and UG2), Negative Control of Extraction, C–, and the Negative Control of Amplification, NCA (DNA-buffer)**.

It is necessary to take reagents for one extra reaction: for N tests, prepare reagents for (N+1) reactions.

4. 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.
5. Prepare the required number of tubes for amplification of DNA from clinical and control samples.
6. Transfer **15 µl** of prepared reaction mixture to the tubes.
7. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage to the prepared tubes using tips with aerosol barrier.

Avoid sorbent adding when transferring the DNA samples.

8. 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 UG1** to the tube labeled UG2.

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

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 for rotor-type instruments[2]

Step	*Temperature, °C*	*Time*	*Fluorescent signal detection*	*Cycle repeats*
Hold	95	15 min	–	1
Cycling	95	5 s	–	5

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

	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	20 s	FAM/Green, JOE/Yellow ROX/Orange, Cy5/Red	
	72	15 s	–	

Table 2

AmpliSens-1 amplification program for plate-type instruments[3]

Step	*Temperature, °C*	*Time*	*Fluorescent signal detection*	*Cycle repeats*
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
3	95	5 s	–	40
	60	30 s	FAM, JOE/HEX, ROX, Cy5	
	72	15 s	–	

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin.*
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

The results are interpreted by the software of the used real-time PCR cycler. Curves of fluorescent signal accumulation are detected in four channels:

- ***U.parvum*** **DNA** amplification product is detected in the **FAM/Green** fluorescence channel,
- ***U.urealyticum*** **DNA** amplification product is detected in the **JOE/Yellow** fluorescence channel,
- ***M. hominis*** **DNA** amplification product is detected in the **ROX/Orange** fluorescence channel,
- **Internal Control DNA (β-globin)** amplification product is detected in the **Cy5/Red** fluorescence channel.

Concentration values of DNA calibrators are specified in the *Important Product Information Bulletin* for each lot of the PCR kit. They should be entered in the corresponding cells of automatic interpretation program.

Results are interpreted by the crossing (or not crossing) of the fluorescence curve with the threshold line set at the level of exponential growth of fluorescence. That determines

[3] For example iCycler iQ5 (Bio-Rad, USA), Mx3000P, Mx3000 (Stratagene, USA).

presence (or absence) of *Ct* (cycle threshold) value of a sample in the appropriate cell of the result grid. Obtained *Ct* values are used for plotting a calibration line and determination of DNA concentration of *U.parvum, U.urealyticum,* and *M.hominis* in clinical samples.

DNA concentrations of *U.parvum, U.urealyticum,* and *M.hominis* can be determined by **AmpliSens® Florocenosis / Mycoplasma-FRT** PCR kit in 2 variants – absolute and normalized values.

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

Absolute concentration of *U.parvum, U.urealyticum* и *M.hominis* shows total content of these microorganisms in clinical material placed in transport media. According to calibrators values initial concentrations (copies/reaction) of *U.parvum, U.urealyticum* and *M.hominis* are automatically calculated. These values are used for calculation of quantity of genome equivalent in 1 ml of clinical sample:

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

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

Normalized concentrations reflect number of microorganism cells relatively to number of human cells. In addition, concentration of human DNA reflects quality of material sampling. According to calibrator values initial concentrations (copies/reaction) of *U.parvum, U.urealyticum, M.hominis* and human DNA are automatically calculated. These values are normalized to 10^5 human cells according to the formula:

$$\frac{\textbf{[Number of copies] DNA } \textbf{\textit{Up (Uu, Mh)}}}{\textbf{[Number of copies] human DNA}} \textbf{ x200,000 = GE } \textbf{\textit{Up (Uu, Mh)}} \textbf{ per } 10^5 \textbf{ human cells}$$

10. TROUBLESHOOTING

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

1. If a signal is detected in FAM/Green, JOE/Yellow, ROX/Orange for negative control of extraction (C–) and/or amplification (NCA). In that case it is necessary to repeat PCR testing for all samples with *Ct* determined in FAM/Green, JOE/Yellow, ROX/Orange channel.
2. If a Calc Conc (copies/reaction) value greater than 5 appears in the results grid for the negative control of extraction (C–) and/or amplification (NCA) in the Cy5/Red channel, it indicates contamination of reagents or samples. In such cases the results of analysis are considered invalid. Test analysis must be repeated (beginning with DNA extraction stage) for those samples that have a signal in the FAM/Green, JOE/Yellow, and

ROX/Orange channel and measures to detect and eliminate the source of contamination must be taken.

3. If a signal is not detected in FAM/Green, JOE/Yellow, ROX/Orange and Cy5/Red for DNA-calibrators (UG1, UG2) or if the difference in *Ct* values is not in the range specified in *Important Product Information Bulletin*. In that case it is necessary to repeat amplification of all samples.
4. If there is no signal for endogenous control (no signal in Cy5/Red channel). In that case it is necessary to repeat clinical material sampling, DNA extraction, and PCR testing.

11. TRANSPORTATION

AmpliSens® Florocenosis / Mycoplasma-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 / 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 expiration date 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 temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FL *U.parvum* / *U.urealyticum* / *M.hominis* is to be kept away from light.

13. SPECIFICATIONS

13.1. 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[4], 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	$1x10^3$

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

Urine	——	DNA-sorb-AM	*Ureaplasma parvum*	$2x10^3$
			Ureaplasma urealyticum	$2x10^3$
			Mycoplasma hominis	$2x10^3$

13.2. Linear range

Linear range for each determining microorganisms is from 10^3 to 10^7 GE/ml.

13.2. 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 is tests of DNA 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, Moscow, 2008.
2. Guidelines “PCR kits for simultaneous qualitative detection and quantitation of STIs in 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” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow.

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.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
RUO	Research use only		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of Amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.08.12 LA	10. Troubleshooting	Mention of Cy5/Red channel was eliminated from the paragraph 1
		Paragraph 2 containing information about criteria of contamination of reagents and samples was added
14.08.13 FN	Principle of PCR detection	Ct was changed to *Ct*
	Footer	Catalogue number **REF** R-B75-100-FT(RG,iQ,Mx)-CE-B was added
21.08.13 FN	Content	One more release form was added AmpliSens® Florocenosis / Mycoplasma-FRT PCR kit variant FRT-100 F in bulk (for use with RG, iQ, Mx), **REF** R-B75-100-FT(RG,iQ,Mx)-CE-B
11.10.13 GA	Text	The name of the kit was changed from “AmpliSens® Florocenosis / Mycoplasma-FRT” to “AmpliSens® Flora-Myco”
	4. Additional requirements	The phrase “deep-freezer at ≤ –16 °C” was changed to “deep-freezer at the temperature from minus 24 to minus 16 °C”
12.11.13 GA	Text	The name of the kit was changed from “AmpliSens® Flora-Myco” to “AmpliSens® Florocenosis / Mycoplasma-FRT”