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For Professional Use Only

AmpliSens® *Ureaplasma* spp.-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***Ureaplasma* spp.-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Ureaplasma* species *(U. parvum and U. urealyticum)* DNA in the clinical materials (urogenital swabs, urine, and prostate gland secretion) 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

Ureaplasma species *(U. parvum and U. urealyticum)* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR 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**® ***Ureaplasma* spp.-FRT** PCR kit is a qualitative test that contains the Internal Control (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**® ***Ureaplasma* spp.-FRT** PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer or a chemically modified polymerase (TaqF). The wax melts and reaction components mix only at 95 °C. Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Ureaplasma* spp.-FRT PCR kit is produced in 3 forms:

AmpliSens® *Ureaplasma* spp.-FRT PCR kit variant FRT (for use with RG),

REF R-B2(RG)-CE.

AmpliSens® *Ureaplasma* spp.-FRT PCR kit variant FRT (for use with iQ),

REF R-B2(iQ)-CE.

AmpliSens® *Ureaplasma* spp.-FRT PCR kit variant FRT-100 F (for use with RG, iQ),

REF R-B2-F(RG,iQ)-CE.

AmpliSens® *Ureaplasma* spp.-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Amount*
PCR-mix-1-FL *Ureaplasma* spp. ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.01	110 tubes of 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	1 tube
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	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-FL during the DNA extraction directly to the sample/lysis mixture (see "DNA-sorb-AM" **REF** K1-12-100-CE protocol).

AmpliSens® *Ureaplasma* spp.-FRT PCR kit is intended for 110 reactions (including controls).

AmpliSens® *Ureaplasma* spp.-FRT PCR kit variant FRT-100 F includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
PCR-mix-1-FL *Ureaplasma* spp.	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
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	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-FL during the DNA extraction directly to the sample/lysis mixture (see the "DNA-sorb-AM" **REF** K1-12-100-CE protocol).

AmpliSens® *Ureaplasma* spp.-FRT PCR kit 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 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); iCycler iQ™ or iQ5™ (Bio-Rad, USA) or equivalent).
- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- 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, and 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 the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. 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 are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® ***Ureaplasma*** **spp.-FRT** PCR kit is intended to analyze DNA extracted with DNA extraction kits from:

- *urogenital swabs,*
- *urine (a sediment of the first portion of the morning specimen),*
- *prostate gland secretion.*

7. WORKING CONDITIONS

AmpliSens® ***Ureaplasma*** **spp.-FRT** PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It's recommended that the following nucleic acid extraction kits are used:

- "DNA-sorb-AM", **REF** K1-12-100-CE.
- Other nucleic acid extraction kits recommended by CRIE.

Extract DNA according to the instructions provided by the manufacturer.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

Variant FRT

Total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

1. Prepare the required number of the tubes with **PCR-mix-1-FL** ***Ureaplasma*** **spp.** and wax for amplification of DNA from clinical and control samples.

2. Add **10 µl** of **PCR-mix-2-FL-red** to the surface of wax layer of each tube, so that it does not fall under the wax and mix with **PCR-mix-1-FL *Ureaplasma* spp.**

Variant FRT-100 F

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

1. Thaw the **PCR-mix-2-FRT** tube. Vortex the tubes with **PCR-mix-1-FL *Ureaplasma* spp., PCR-mix-2-FRT,** and **polymerase (TaqF)** then centrifuge briefly.
   Collect the required number of the tubes/stripes for amplification of DNA obtained from clinical and control samples.
2. For N reactions (including 2 controls) mix in a new tube:
   10*(N+1) µl of **PCR-mix-1-FL *Ureaplasma* spp.;**
   5.0*(N+1) µl of **PCR-mix-2-FRT;**
   0.5*(N+1) µl of **polymerase (TaqF).**
   Vortex the tube, then centrifuge briefly. Transfer **15 µl** of the prepared mixture to each tube.

Steps 3 and 4 are carried out in both variants.

3. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage into the prepared tubes using tips with aerosol barrier.
4. Carry out the 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 complex** (to the tube labeled C+ (Positive Control of Amplification).

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

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 program

	Rotor-type Instruments[1]			Plate-type Instruments[2]		
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 *fluorescent signal detection*		**60**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

Fluorescence detection should be enabled in the channels designed for the FAM and JOE fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).

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

9. DATA ANALYSIS

The fluorescent signal intensity is detected in two channels:

- The signal from the *Ureaplasma* spp*.* DNA amplification product is detected in the FAM channel;
- The signal from the Internal Control amplification product is detected in the JOE channel.

Result interpretation

The results are interpreted with the Instrument software by the crossing (or not-crossing) of the fluorescence curve with a threshold line and it is showed as presence (or absence) of Ct (threshold cycle) in the result grid.

Principle of interpretation:

- *Ureaplasma* spp. DNA is **detected** in a sample if its Ct is defined in the result grid in the FAM channel. Moreover, the fluorescence curve should cross the threshold line in the area of exponential fluorescence growth.
- *Ureaplasma* spp. DNA is **not detected** in a sample if its Ct is not defined in the result grid in the FAM channel (the fluorescence curve does not cross the threshold line) whereas Ct in the JOE channel is less than the specified boundary value.
- The result is **invalid** if Ct of a sample in the FAM channel is absent whereas Ct in the

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[2] For example, iCycler iQ, iQ5, Mx3000P, Mx3000, DT-96 or equivalent.

JOE channel is either absent or greater than the specified boundary value. It is necessary to repeat the PCR test for such a sample.

Ct boundary values are specified in the ***Important Product Information Bulletin*** enclosed to the PCR kit.

The result of the analysis is considered reliable only if the results of both Positive and Negative Controls of amplification as well as Negative Control of extraction are correct (Table 2).

Table 2

Results for controls

Control	Stage for control	Ct value in channel		Interpretation
		FAM fluorophore	JOE fluorophore	
C-	DNA extraction	Neg	Pos (< boundary value*)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< boundary value*)	Pos (< boundary value*)	OK

For boundary values, see the ***Important Product Information Bulletin*** enclosed to the PCR kit.

10. TROUBLESHOOTING

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

- If the Ct value is absent in both JOE/Yellow/HEX and FAM/Green channels or the Ct value in the JOE/Yellow/HEX channel is greater than the specified boundary value, PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (if iCycler iQ or iQ5 are used).
- If the Ct value is present for C– in the FAM/Green channel and/or for NCA in the FAM, JOE/Yellow/HEX channels in the results grid, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
- If no signal is detected for the Positive Control of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components has not complied with the manufacturer's instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be

repeated.

- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if Cycler iQ or iQ5 instruments are used).

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

11. TRANSPORTATION

AmpliSens® *Ureaplasma* spp.-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® *Ureaplasma* spp.-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Ureaplasma* spp.-FRT** PCR kit are stable until the labeled expiration date. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FL *Ureaplasma* spp. should be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Ureaplasma* spp.-FRT** PCR kit is specified in the table below.

Clinical material	DNA extraction kit	Analytical sensitivity, GE/ml[3]
Urogenital swabs[4]	"DNA-sorb-AM"	1 x 10^3
Urine (pretreatment is required)[5]	"DNA-sorb-AM"	2 x 10^3

13.2. Specificity

The analytical specificity of **AmpliSens® *Ureaplasma* spp.-FRT** PCR kit is ensured by selection of specific primers and probes as well as by selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. There were not nonspecific test responses during examination of a human DNA as well as a DNA panel of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Mycoplasma hominis, Mycoplasma genitalium, Candida albicans, Neisseria flava, Neisseria subflava, Neisseria sicca, Neisseria mucosa, Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* types 1 and 2, *CMV, and HPV.*

The clinical specificity of **AmpliSens® *Ureaplasma* spp.-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 "Real-Time PCR Detection of STIs and Other Reproductive Tract Infections", 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.

[3] Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

[4] Urogenital swabs are to be placed into the Transport Medium for Swabs (**REF** 956-CE, 987-CE) or Transport Medium with Mucolytic Agent (**REF** 953-CE).

[5] Pretreatment is required.

15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of **AmpliSens®** ***Ureaplasma*** **spp.-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	**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
29.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”