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For Professional Use Only

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***C.trachomatis / Ureaplasma / M.genitalium / M.hominis*****-MULTIPRIME-FRT** PCR kit is an *in vitro* nucleic acid amplification test for multiplex detection of DNA of *Chlamydia trachomatis*, *Ureaplasma (U.parvum* and *U.urealyticum)*, *Mycoplasma genitalium,* and *Mycoplasma hominis* in clinical materials (urogenital, rectal and pharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) 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

Chlamydia trachomatis / *Ureaplasma* / *Mycoplasma genitalium* / *Mycoplasma hominis* detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific regions using specificl *Chlamydia trachomatis* / *Ureaplasma* / *Mycoplasma genitalium* / *Mycoplasma hominis* 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 monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens®** ***C.trachomatis / Ureaplasma / M.genitalium / M.hominis*****-MULTIPRIME-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®** ***C.trachomatis / Ureaplasma / M.genitalium / M.hominis*****-MULTIPRIME-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 a wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 ºC. Chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min.

3. CONTENT

AmpliSens® ***C.trachomatis / Ureaplasma / M.genitalium / M.hominis*****-MULTIPRIME-FRT** PCR kit is produced in 2 forms:

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*-MULTIPRIME-FRT PCR kit variant FRT, **REF** R-B60(RG)-CE.

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*-MULTIPRIME-FRT PCR kit variant FRT-100 F, **REF** R-B60-F(RG)-CE.

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium / M.hominis* (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 (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see “DNA-sorb-AM” **REF** K1-12-100-CE protocol).

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*-MULTIPRIME-FRT PCR kit, variant FRT is intended for 110 reactions, including controls.

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-FRT PCR kit, variant FRT-100 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*	colorless clear liquid	1.1	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	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 procedure directly to the sample/lysis mixture (see “DNA-sorb-AM” **REF** K1-12-100-CE protocol).

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*-MULTIPRIME-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 6000 (Corbett Research, Australia), Rotor-Gene Q (Qiagen, Germany), or equivalent).
- Disposable polypropylene microtubes for PCR (0.2- or 0.1-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 mucous membranes. If skin, eyes, or mucous

membranes contact, immediately flush with water and 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 is described in manufacturer's handbook [1]. It is recommended to read this handbook before starting work.

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*-MULTIPRIME-FRT PCR kit is intended for analysis of DNA extracted by using DNA extraction kits from

- urogenital swabs;
- rectal swabs;
- pharyngeal swabs;
- conjunctival discharge and prostate gland secretion;
- urine samples (use the first part of the stream).

7. WORKING CONDITIONS

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*-MULTIPRIME-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.
- Other nucleic acid extraction kits recommended 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].

Extract DNA according to the manufacturer's instructions.

8.2. Preparing the PCR

Variant FRT

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

8.2.1. Preparing tubes PCR

1. Prepare the required number of the tubes with **PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*** 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 the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium / M.hominis***.

Variant FRT-100 F

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

1. Prepare the required number of the tubes for amplification of DNA from clinical and control samples (0.2-ml tubes for a 36-well rotor or 0.1-ml strips for a 72-well rotor).
2. For carrying out N reactions (including 2 controls), mix in a new tube: **10*(N+1) µl of PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*, 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 the prepared mix to each tube.

Steps 3 and 4 are applicable to both variants.

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

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).

8.2.2. Amplification

Program the thermocycler according to **Manufacturer's manual**, **Guidelines** and Table 1.

Table 1

AmpliSens-1 RG amplification program

Step	***Temperature, °C***	***Time***	***Cycle repeats***
Hold	95	15 min	1
Cycling	95	5 s	5
	60	20 s	
	72	15 s	
Cycling 2	95	5 s	40
	60	**20 s (fluorescence detection)**	
	72	15 s	

Fluorescence is detected at the 2-nd step of stage Cycling 2 **(60 °C)** in Green, Yellow, Orange, Crimson, and Red fluorescence channels.

9. DATA ANALYSIS

***Chlamydia trachomatis* DNA** amplification product is detected in the **Green** fluorescence channel, ***Ureaplasma* DNA** is detected in the **Yellow** channel, ***Mycoplasma genitalium* DNA** is detected in the **Orange** channel, ***Mycoplasma hominis* DNA** is detected in the **Crimson** channel, and **Internal Control DNA** is detected in the **Red** channel.

Interpretation of results

The results are interpreted by the software of the PCR instrument used by the crossing (or not crossing) of the fluorescence curve with the threshold line.

The results of analysis are considered reliable only if the results obtained for both Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct.

Table 2

Results for controls

Control	Stage for control	Ct channels Green, Yellow, Orange, Crimson	Ct channel Red	Interpretation
C–	DNA extraction	Neg	Pos (< boundary value) *	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< boundary value) *	Pos (< boundary value) *	OK

1. The sample is considered to be positive for *Chlamydia trachomatis* if its Ct value is detected in the results grid (the fluorescence curve crosses the threshold line) in the Green channel.
2. The sample is considered to be positive for *Ureaplasma* if its Ct value is detected in the results grid (the fluorescence curve crosses the threshold line) in the Yellow channel.
3. The sample is considered to be positive for *Mycoplasma genitalium* if its Ct value is detected in the results grid (the fluorescence curve crosses the threshold line) in the Orange channel.
4. The sample is considered to be positive for *Mycoplasma hominis* if its Ct value is detected in the results grid (the fluorescence curve crosses the threshold line) in the Crimson channel.
5. The sample is considered to be negative for *Chlamydia trachomatis*, *Ureaplasma, Mycoplasma genitalium,* and *Mycoplasma hominis* if its Ct value is not detected in the results grid (the fluorescence curve does not cross the threshold line) in Green, Yellow, Orange and Crimson channels and the Ct value does not exceed the boundary Ct value in the results grid in the Red channel.

* For Ct boundary values of the samples, Negative Control of Extraction and Positive Control of Amplification, see **Appendix 1**.

10. TROUBLESHOOTING

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

- If no signal is detected for the sample or its Ct value exceeds the boundary value in Green, Yellow, Orange, Crimson and Red channels, PCR should be repeated. If the same result is obtained, the sample analysis should be repeated starting from the extraction stage.
- If no signal is detected for Positive Control of Amplification (C+) or its Ct value exceeds boundary value in Green, Yellow, Orange and Crimson channels, PCR reaction should be repeated for the samples without detected signal in the channels.
- If the positive signal in negative controls (C- or NCA) in the channels for detection of pathogen DNA is registered, analysis must be repeated for the samples for which Ct value is defined.
- If no signal was detected in the channels for detection of pathogen DNA and for detection of Internal Control, the result is considered to be invalid. The sample should be examined again (PCR and detection).

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

11. TRANSPORTATION

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-FRT** PCR kit are stable until expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium / M.hominis* is to be kept away from light.

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

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity for *Chlamydia trachomatis*, *Ureaplasma*, *Mycoplasma genitalium,* and *Mycoplasma hominis* is not less than $5x10^2$ genome equivalents per 1 ml of sample (GE/ml).

The analytical sensitivity of each microorganism does not change even at high concentrations of the three other microorganisms.

13.2. Specificity

The analytical specificity of **AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The clinical specificity of **AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-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.

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® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-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
RG	Rotor-Gene	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”