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For Professional Use Only

AmpliSens® *C.trachomatis / Ureaplasma*-MULTIPRIME-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® ***C.trachomatis / Ureaplasma*-MULTIPRIME-FEP** PCR kit is an *in vitro* nucleic acid amplification test for multiplex detection of *Chlamydia trachomatis* and *Ureaplasma* spp. DNA in clinical materials (urogenital, rectal and pharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) by using end-point hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

C.trachomatis / *Ureaplasma* spp. detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *C.trachomatis* / *Ureaplasma* spp. primers. In **F**luorescent **E**nd-**P**oint PCR, the amplified product is detected by using fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescence emission from the fluorophores in the reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *C.trachomatis / Ureaplasma*-MULTIPRIME-FEP** PCR kit is a qualitative test that contains the Internal Control. 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*-MULTIPRIME-FEP** 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. Wax melts and reaction components mix only at 95 °C.

3. CONTENT

AmpliSens® *C.trachomatis / Ureaplasma*-MULTIPRIME-FEP PCR kit is produced in 2 forms:

AmpliSens® *C.trachomatis* / *Ureaplasma*-MULTIPRIME-FEP PCR kit variant FEP (0.5-ml tubes),

REF B47-100-R0,5-FEP-CE.

AmpliSens® *C.trachomatis* / *Ureaplasma*-MULTIPRIME-FEP PCR kit variant FEP (0.2-ml tubes),

REF B47-100-R0,2-FEP-CE.

AmpliSens® ***C.trachomatis* / *Ureaplasma*-MULTIPRIME-FEP** PCR kit variant FEP includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FL *C.trachomatis* / *Ureaplasma*	colorless clear liquid	0.01	110 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	1 tube
Mineral oil for PCR*	colorless viscous liquid	4.0	1 dropper bottle
PCR-mix-Background-red	red clear liquid	0.6	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 for thermocyclers without a constant-temperature lid.

** 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 (DNA-sorb-AM, **REF** K1-12-100-CE).

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

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves and laboratory coat.
- Adjustable pipettes.
- 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, Terzik (DNA-Technology), Gradient Palm Cycler (Corbett Research, Australia), MaxyGene (Axygen, USA), GeneAmp PCR System 2700 (Applied Biosystems, USA), or equivalent).
- Fluorometer (fluorescence detector; for example, ALA-1/4 (Biosan, Latvia) 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 samples or reagent spills using a disinfectant such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucous membranes. If skin, eyes, or and mucous membranes 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 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 manufacturer's handbook [1]. It is recommended to read this handbook before starting work.

AmpliSens® *C.trachomatis* / *Ureaplasma*-MULTIPRIME-FEP 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*-MULTIPRIME-FEP 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 (see manufacturer's **Guidelines**).

Extract DNA according to the manufacturer's instructions.

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

1. Prepare the required number of tubes with **PCR-mix-1-FL *C.trachomatis / Ureaplasma*** 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 in each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *C.trachomatis / Ureaplasma***.
3. Add above **1** drop of **mineral oil for PCR** (about **25 µl**).

4. Prepare 1 tube with **PCR-mix-1-FL *C.trachomatis / Ureaplasma*** and mark them as **Background**. Add **20 µl** of **PCR-mix-Background-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***. Add above **1** drop of **mineral oil for PCR**.

Use **mineral oil for PCR** if working with thermocyclers without a constant-temperature lid.

PCR-mix-Background-red is to be used if DNA was extracted using DNA-sorb-AM, **REF** K1-12-100-CE, or DNA-sorb-B, **REF** K1-2-100-CE. See the manufacturer's instruction if other nucleic acid extraction kits were used.

5. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage.
6. 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).

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them in the thermocycler.

8.2.2 Amplification

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95 °C (pause regimen), insert tubes to cells of amplifier and press the button to continue.

Table 1

Amplisens-1-FEP amplification program

	Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems)**			**Gradient Palm Cycler (Corbett Research), MaxyGene (Axygen)**		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
0	**95**	pause		**95**	pause		**95**	pause	
1	**95**	5 min	1	**95**	5 min	1	**95**	5 min	1
2	**95**	2 s	35	**95**	20 s	20	**95**	2 s	24
	65	5 s		**65**	25 s		**65**	10 s	
	72	5 s		**72**	30 s		**72**	10 s	
3	**95**	2 s	9	**95**	20 s	24	**95**	2 s	20
	60	10 s		**60**	30 s		**60**	15 s	
	72	5 s		**72**	30 s		**72**	10 s	
4	**95**	2 s	1	**95**	20 s	1	**95**	2 s	1
	60	10 s		**60**	30 s		**60**	15 s	
5	**10**	storage		**10**	storage		**10**	storage	

Programming for thermocyclers is described in manufacturer's guidelines [2] as well. It is recommended to read these guidelines before starting work.

9. DATA ANALYSIS

Detection is conducted using a fluorescence detector.

Please read the fluorescence detector Operating Manual before use of this kit.

Program the detector according to the **Manufacturer's manual** and **Important product information bulletin**.

Accumulation of ***Chlamydia trachomatis*** **DNA** amplification product is detected in the **FAM/Green** fluorescence channel, ***Ureaplasma*** **spp. DNA** is detected in the **HEX/Yellow** channel, Internal Control (IC) is detected in the **ROX/Orange** channel.

9.1 Results interpretation

1. Principle of interpretation:

— *Chlamydia trachomatis* DNA is **detected** in a sample if its signal in the FAM channel is greater than the defined threshold value of the positive result.

— *Ureaplasma spp*. DNA is **detected** in a sample if its signal in the HEX channel is greater than the defined threshold value of the positive result.

— *Chlamydia trachomatis* DNA and *Ureaplasma* spp. DNA are **not detected** in a sample if their signals in the FAM and HEX channels are less than the defined threshold value of the negative result while the signal in the ROX channel is greater than the threshold value defined for this channel.

— The result is **invalid** if the signal of a sample in FAM, HEX, and ROX channels is less than the threshold values defined for these channels.

— The result is **equivocal** if the signal of a sample in the FAM channel is greater than the defined threshold value of the negative result but less than the threshold value of the positive result (the signal is between thresholds).

If the result is invalid or equivocal, the PCR should be repeated once again.

2. The result of analysis is considered reliable only if the results obtained for 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	Result of automatic interpretation			Interpretation
		FAM channel (samples)	**HEX channel** (samples)	**ROX channel** (IC)	
C-	DNA extraction	**<** threshold of negative result	**<** threshold of negative result	**>** threshold	"-" or "OK"
NCA	Amplification	**<** threshold of negative result	**<** threshold of negative result	**<** threshold	"nd"
C+	Amplification	**>** threshold of positive result	**>** threshold of positive result	**>** threshold	"+" or "OK"

10. TROUBLESHOOTING

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

- If the signal of the Positive Control of Amplification (C+) in FAM and HEX channels is less than the threshold of the positive result, repeat PCR and detection for all samples in which the signal was less than the threshold of the positive result.
- If the signal of the Negative Control of Extraction (C–) and/or Negative Control of Amplification (NCA) in FAM and/or HEX channels is greater than the threshold of the positive result, repeat analysis of all samples in which the signal was greater than the threshold of the positive result starting from the extraction stage.

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*-MULTIPRIME-FEP 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*-MULTIPRIME-FEP** PCR kit are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *C.trachomatis / Ureaplasma*-MULTIPRIME-FEP** 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-1-FL *C.trachomatis / Ureaplasma* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Nucleic acid extraction kit	PCR kit	Microorganism	Sensitivity, GE/ml [1]
Cervical or urethral swabs [2]	DNA-sorb-AM	PCR kit variant FEP	*Chlamydia trachomatis*	$5x10^2$
			Ureaplasma spp.	10^3
Urine [3]	DNA-sorb-AM	PCR kit variant FEP	*Chlamydia trachomatis*	10^3
			Ureaplasma spp.	$2x10^3$

The analytical sensitivity of each microorganism (up to 10^9 GE/ml) does not change even if the other microorganism is present at a high concentration.

13.2. Specificity

The analytical specificity of **AmpliSens® *C.trachomatis / Ureaplasma*-MULTIPRIME-FEP** PCR kit is ensured by 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. The clinical specificity of **AmpliSens® *C.trachomatis / Ureaplasma*-MULTIPRIME-FEP** PCR kit was confirmed in laboratory clinical trials.

Nonspecific responses were absent in tests of human DNA samples and DNA samples of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Chlamydia trachomatis, Mycoplasma genitalium, Neisseria* spp., *Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* types 1 and 2, *CMV,* and *HPV*.

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 “End-Point 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.

[1] The quantity of genome equivalents of microorganism per 1 ml of the sample from transport medium.

[2] Cervical or urethral swabs are to placed into Transport Medium for Swabs (**REF** 956-CE, 987-CE) or Transport Medium with Mucolytic Aagent (**REF** 952-CE, 953-CE).

[3] Pretreatment is required.

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*-MULTIPRIME-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

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”