



For *in Vitro* Diagnostic Use

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# AmpliSens® *C. trachomatis /Ureaplasma-*

## MULTIPRIME-FEP

### PCR kit

### Instruction Manual

# AmpliSens®



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## 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 the clinical materials (urogenital swabs, rectum swabs, pharynx mucous membrane, urine sediment, conjunctiva samples, secrete of the prostate gland) 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 Fluorescent End-Point PCR, the amplified product is detected by using fluorescent dyes. These dyes are usually 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 a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens® C. trachomatis /Ureaplasma-MULTIPRIME-FEP** PCR kit is a qualitative test, which contains the Internal Control. It must be used in the isolation procedure in order to control the isolation 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 frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using 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.

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

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

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

Reagent	Description	Volume (ml)	Quantity
<b>PCR-mix-1-FL C.trachomatis / Ureaplasma</b>	colorless, clear liquid	0.01	110 tubes of 0.5 or 0.2 ml
<b>PCR-mix-2-FL-red</b>	red clear liquid	1.1	1 tube
<b>Mineral oil for PCR</b>	colorless, viscous liquid	4.0	1 dropper bottle
<b>PCR-mix-Background-red</b>	colorless, clear liquid	0.6	1 tube
<b>Positive Control complex (C+)</b>	colorless, clear liquid	0.2	1 tube
<b>DNA-buffer</b>	colorless, clear liquid	0.5	1 tube
<b>Negative Control (C-)*</b>	colorless, clear liquid	1.2	1 tube
<b>Internal Control-FL (IC)**</b>	colorless, clear liquid	1.0	1 tube

\* must be used in the isolation procedure as Negative Control of Extraction.

\*\* add 10 µl of Internal Control-FL (IC) during the DNA isolation 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 isolation 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, Terzik (DNA-Technology), Gradient Palm Cycler (Corbett Research, Australia), MAXYGENE (Axigen, 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 with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator for temperature 2-8 °C.
- Deep-freezer with temperature not more than minus 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 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 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 isolation kits from:

- cervical or urethral scrapes (swabs),
- rectum swabs,
- pharynx mucous membrane,
- urine sediment (use the first part of the stream),
- conjunctiva samples or secrete of the prostate gland.

## 7. PROTOCOL

### 7.1. DNA Isolation

It's 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 State Institution of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being (see manufacturer's **Guidelines**).



Carry out the DNA isolation according to the manufacturer's instructions.

### 7.2. Preparing the PCR

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

#### 7.2.1 Preparing tubes for PCR

- Prepare the required number of tubes with **PCR-mix-1-FL *C.trachomatis* / *Ureaplasma*** for amplification of DNA from clinical and control samples.
- 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***.
- Add above **1** drop of **mineral oil for PCR** (about **25 µl**).
- 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**.



PCR-mix-Background-red is to be used if DNA was isolated 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.

- Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage.
- 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 vortexing (1–3 sec) before placing them in the thermocycler.

#### 7.2.2 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

Step	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (Applied Biosystems)			"Gradient Palm Cycler" (Corbett Research), "MAXYGENE" (Axygen)		
	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 sec	35	95	20 sec	20	95	2 sec	24
	65	5 sec		65	25 sec		65	10 sec	
	72	5 sec		72	30 sec		72	10 sec	
3	95	2 sec	9	95	20 sec	24	95	2 sec	20
	60	10 sec		60	30 sec		60	15 sec	
	72	5 sec		72	30 sec		72	10 sec	
4	95	2 sec	1	95	20 sec	1	95	2 sec	1
	60	10 sec		60	30 sec		60	15 sec	
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.

## 8. 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 **JOE/Yellow/HEX** channel, Internal Control (IC) is detected in the **ROX/Orange** channel.

## Results interpretation

1. Principle of interpretation:

- *Chlamydia trachomatis* DNA is **detected** in a sample if its signal in the FAM channel is more 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 more 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 more than the defined threshold value.

- The result is **invalid** if the signal of a sample in FAM, HEX and ROX channels is less than defined threshold values for these channels.
- The result is **equivocal** if the signal of a sample in the FAM channel is more 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. Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (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 isolation	< threshold of negative result	< threshold of negative result	> threshold	"-" or "OK"
NCA	Amplification	< threshold of negative result	< threshold of negative result	< threshold	"nd" or "OK"
C+	Amplification	> threshold of positive result	> threshold of positive result	> threshold	"+" or "OK"

## 9. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

- No signal with the positive control of PCR (C+) may indicate incorrect programming of the temperature profile of the thermocycler or incorrect configuration of the PCR reaction or storage conditions for kit components did not comply with the manufacturer's instruction or the reagents kit had expired. It is necessary to check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR once again.
- Positive signal in negative controls (C- or NCA) indicates the reagent or sample contamination. In such cases the results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting the contamination source must be undertaken.
- If no signal was detected in the channels for detection of the pathogen DNA and for detection of the Internal Control, the sample should be examined repeatedly (PCR and detection). The same procedures should be performed for the samples with equivocal result, that is, specific signal exceeds the background not enough to consider the sample as positive.

## 10. STABILITY AND STORAGE

All components of the **AmpliSens® C. trachomatis /Ureaplasma-MULTIPRIME-FEP** PCR kit are to be stored at the temperature 2-8 °C, when not in use. All components of the **AmpliSens® C. trachomatis /Ureaplasma-MULTIPRIME-FEP** PCR kit are to be stable until the expiration date.



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

## 11. SPECIFICATIONS

### 11.1. Sensitivity

Clinical material	Nucleic acid extraction kit	PCR kit	Microorganism	Sensitivity, GE/ml <sup>1</sup>
Cervical, urethral scrapes (swabs) <sup>2</sup>	DNA-sorb-AM	PCR kit variant FEP	<i>Chlamydia trachomatis</i>	5x10 <sup>2</sup>
			<i>Ureaplasma spp.</i>	10 <sup>3</sup>
Urine <sup>3</sup>	DNA-sorb-AM	PCR kit variant FEP	<i>Chlamydia trachomatis</i>	10 <sup>3</sup>
			<i>Ureaplasma spp.</i>	2x10 <sup>3</sup>



Analytical Sensitivity of each microorganism doesn't change even at high concentrations of other microorganisms.

### 11.2. Specificity

Specificity of **AmpliSens® C. trachomatis /Ureaplasma-MULTIPRIME-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. Specificity of **AmpliSens® C. trachomatis /Ureaplasma-MULTIPRIME-FEP** PCR kit was confirmed in laboratory clinical trials.

Nonspecific responses were absent while testing human DNA samples and DNA samples of 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 1 and 2*, *CMV*, *HPV*.

<sup>1</sup> The quantity of genome equivalents of microorganism per 1 ml of the sample from transport medium.

<sup>2</sup> Cervical, urethral scrapes (swabs) are to be placed into Transport medium for swabs (REF 956, 987) or Transport medium with mucolytic (REF 952, 953).

<sup>3</sup> Treatment is needed.

## 12. REFERENCES

- Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", issued by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.
- Guidelines "End-point PCR detection of STIs and other reproductive tract infections", issued by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow.

## 13. QUALITY CONTROL

In compliance with Federal State Institution of Science Central Research Institute of Epidemiology ISO 13485 – certified Quality Management System, each lot of **AmpliSens® C. trachomatis /Ureaplasma-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

## 14. EXPLANATION OF SYMBOLS



Manufacturer



Temperature limitation



Use by



Batch code



For *in Vitro* Diagnostic Use



Version



Catalogue number



Internal Control complex



Contains sufficient for <n> tests



Authorized representative in the European Community.



Consult instructions for use



Caution, consult accompanying documents



For working with Rotor-Gene™ 3000/6000



For working with iQ5, iQ iCycler



Positive control



Negative control