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For Professional Use Only

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

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

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1. INTENDED USE

AmpliSens® ***C.trachomatis / Ureaplasma / M.hominis*****-MULTIPRIME-FEP** PCR kit is an *in vitro* nucleic acid amplification test for multiplex detection of DNA of *Chlamydia trachomatis, Ureaplasma* (*U.parvum* and *U.urealyticum*) and *Mycoplasma hominis* 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* / *M.hominis* detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *C.trachomatis* / *Ureaplasma* / *M.hominis* 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 fluorescent emission from the fluorophores in a reaction mix after PCR. It allows detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens®** ***C.trachomatis / Ureaplasma / M.hominis*****-MULTIPRIME-FEP** 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.hominis*****-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 ºC.

3. CONTENT

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

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.hominis*-MULTIPRIME-FEP PCR kit variant FEP (0.5 ml volume tubes), **REF** B43-100-R0,5-FEP-CE.

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.hominis*-MULTIPRIME-FEP PCR kit variant FEP (0.2 ml volume tubes), **REF** B43-100-R0,2-FEP-CE.

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

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.hominis* ready-to-use single-dose test tubes (*under wax)*	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 / M.hominis*-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.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a 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)б 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 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.hominis*-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 / M.hominis*-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 **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 / M.hominis*** 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.hominis***.
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 / M.hominis*** 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* / *M.hominis***. Add above **1** drop of **mineral oil for PCR**.

PCR-mix-Background-red is to be used if DNA was isolated by 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 spin down drops from walls of tubes by short vortexing (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 ºC (pause mode), insert tubes into cells of the thermocycler, 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 other thermocyclers is described in manufacturer's guidelines [2]. It is recommended to read this handbook before starting work.

9. DATA ANALYSIS

Detection is conducted using a fluorescence detector.

Please read the fluorescence detector Operating Manual before using this kit.

Program the detector according to the manufacturer's manual and *Important Product Information Bulletin*.

Chlamydia trachomatis **DNA** amplification product is detected in the **FAM/Green** fluorescence channel, ***Ureaplasma*** **spp.** *(**U.parvum*** and ***U.urealyticum**)* **DNA** is detected in the **JOE/Yellow/HEX** channel, ***Mycoplasma hominis*** **DNA** is detected in the **ROX/Orange** channel, and the Internal Control-FL (IC) is detected in the **Cy5/Red** 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. (*U.parvum* and *U.urealyticum)* DNA is **detected** in a sample if its signal in the HEX channel is greater than the defined threshold value.

— *Mycoplasma hominis* DNA is **detected** in a sample if its signal in the ROX channel is gereater than the defined threshold value of the positive result.

— *Chlamydia trachomatis* DNA, *Ureaplasma* spp. DNA and *Mycoplasma hominis* DNA are **not detected** in a sample if their signals in FAM and HEX channels are less than the defined threshold value of the negative result and the signal in the Cy5 channel is greater than the defined threshold value.

— The result is **invalid** if the signal of a sample in FAM, HEX, ROX, and Cy5 channels is less than the defined threshold values for these channels.

— The result is **equivocal** if the signal of a sample in the FAM and/or ROX channel(s) 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. 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** (samples)	**Cy5 channel** (IC)	
C–	DNA extraction	**<** threshold of negative result	**<** threshold	**<** threshold of negative result	**>** threshold	"-" or "OK"
NCA	Amplification	**<** threshold of negative result	**<** threshold	**<** threshold of negative result	**<** threshold	"nd"
C+	Amplification	**>** threshold of positive result	**>** threshold	**>** threshold of positive result	**>** threshold	"+" or "OK"

10. TROUBLESHOOTING

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

- If the signal of C+ in FAM, and/or HEX, and/or ROX channels is less than the threshold of the positive result, repeat PCR and detection for all samples in which the signal in FAM, and/or HEX, and/or ROX channels was less than the threshold of the positive result.
- If the signal of C– and/or NCA in the FAM, and/or HEX, and/or ROX channels is greater than the threshold of the positive result, run PCR starting from the extraction stage for all samples in which the signal was greater than the threshold of the positive result.

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.hominis*-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 / M.hominis*-MULTIPRIME-FEP** PCR kit are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *C.trachomatis / Ureaplasma / M.hominis*-MULTIPRIME-FEP** PCR kit are to be 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.hominis* 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]
Urogenital swabs [2]	DNA-sorb-AM	PCR kit variant FEP	*Chlamydia trachomatis*	$5x10^2$
			Ureaplasma spp.	10^3
			Mycoplasma hominis	10^3
Urine [3]	DNA-sorb-AM	PCR kit variant FEP	*Chlamydia trachomatis*	10^3
			Ureaplasma spp.	$2x10^3$
			Mycoplasma hominis	$2x10^3$

The analytical sensitivity of each microorganism does not change even if two other microorganisms are present at high concentrations.

13.2. Specificity

The analytical specificity of **AmpliSens® *C.trachomatis / Ureaplasma / M.hominis*-MULTIPRIME-FEP** 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.hominis*-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 State Institute of Science “Central Research Institute of 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

[1] The quantity of genome equivalents of microorganism per 1 ml of the sample from transport medium.
[2] Cervical, urethral scrapes (swabs) are to placed into Transport medium for swabs (**REF** 956-CE, 987-CE) or Transport medium with mucolytic (**REF** 952-CE, 953-CE).
[3] Treatment is needed.

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.hominis*-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”