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For Professional Use Only

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-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.genitalium*-MULTIPRIME-FEP** PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of DNA of *Chlamydia trachomatis, Ureaplasma* spp. (*U.parvum* and *U.urealyticum*), and *Mycoplasma genitalium* in the clinical material (urogenital, rectal and oropharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) by 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

Chlamydia trachomatis, Ureaplasma spp. *(U.parvum* and *U.urealyticum), Mycoplasma genitalium* detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of a pathogen genome specific region using specific primers. In Fluorescent End-Point PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescent emission from the fluorophores in the reaction mixture after the PCR. It allows the detection of the accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® ***C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FEP** PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (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*-MULTIPRIME-FEP** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the 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* / *M.genitalium*-MULTIPRIME-FEP** PCR kit is produced in 2 forms:

AmpliSens® ***C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FEP** PCR kit variant FEP (0.5 ml tubes), **REF** B46-100-R0,5-FEP-CE.

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FEP PCR kit variant FEP (0.2 ml tubes), **REF** B46-100-R0,2-FEP-CE.

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

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium* 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.

** is used if DNA samples were extracted using DNA-sorb-AM or DNA-sorb-B kits.

*** 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*-MULTIPRIME-FEP PCR kit is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with filter (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.

- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), MaxyGene (Axygen, USA), GeneAmp PCR System 2700 (Applied Biosystems, USA)).
- Fluorometer ALA-1/4 (BioSan, Latvia) or equivalent instrument.
- Refrigerator for 2–8 °C.
- Deep-freezer for the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge them briefly.
- Use disposable protective gloves, laboratory coats, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- 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 the local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area where 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 the 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® *C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FEP PCR kit is intended for analysis of DNA extracted with the use of DNA extraction kits from the clinical material (urogenital, rectal, and oropharyngeal swabs; conjunctival discharge; urine (a sediment of the first portion of the morning specimen), prostate gland secretion).

7. WORKING CONDITIONS

AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FEP PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

It's recommended to use the following nucleic acid extraction kits:

- DNA-sorb-AM, **REF** K1-12-100-CE.
- For other nucleic acid extraction kits see Guidelines [2].

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL (IC).**

Extract the DNA according to the manufacturer's protocol.

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

The type of tubes depends on the PCR instrument used for analysis.

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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

3. Add above **1** drop of **mineral oil for PCR** (if using thermocyclers without a constant-temperature lid).
4. Prepare 1 tube with **PCR-mix-1-FL *C.trachomatis* / *Ureaplasma* / *M.genitalium*** and mark it as **Background**. Add **20 µl** of **PCR-mix-Background-red** to the surface of the wax layer of the tube, ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *C.trachomatis* / *Ureaplasma* / *M.genitalium***. Add above **1** drop of **mineral oil for PCR** (if using thermocyclers without a constant-temperature lid).

Use **PCR-mix-Background-red** reagent only if DNA samples were extracted using DNA-sorb-AM **REF** K1-12-100-CE or DNA-sorb-B **REF** K1-2-100-CE kits. If any other nucleic acid extraction kits (recommended by FBIS CRIE) were used, follow the instructions provided by the manufacturer.

5. Using filter tips add **10 µl** of **DNA samples** obtained 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 (C+)** to the tube labeled C+ (Positive Control of Amplification).

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

8.2.2 Amplification

1. Run the following program in the thermocycler (see Table 1).
2. When the temperature reaches 95 °C (pause mode), insert tubes into the wells of the thermocycler and press the button to continue.

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

Table 1

Amplisens-1-FEP amplification program

	GeneAmp PCR System 2700			**Gradient Palm Cycler, MaxyGene**		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
0	**95**	pause		**95**	pause	
1	**95**	5 min	1	**95**	5 min	1
2	**95**	20 s	20	**95**	2 s	24
	65	25 s		**65**	10 s	
	72	30 s		**72**	10 s	
3	**95**	20 s	24	**95**	2 s	20
	60	30 s		**60**	15 s	
	72	30 s		**72**	10 s	
4	**95**	20 s	1	**95**	2 s	1
	60	30 s		**60**	15 s	
5	**10**	storage		**10**	storage	

Note – Amplification programs for some other models of thermocyclers are specified in

Guidelines [2].

3. Proceed to fluorescence detection after the amplification program is completed.

9. DATA ANALYSIS

Please read the ALA-1/4 Operating Manual before using this kit.

The detection is performed by means of a fluorescence detector by measuring the fluorescence signal intensity in three channels:

– The channel for the FAM fluorophore (FAM channel or analogous, depending on the detector model) is intended for the detection of the signal of the *Chlamydia trachomatis* DNA amplification product.
– The channel for the JOE fluorophore (HEX channel or analogous, depending on the detector model) is intended for the detection of the signal of the *Ureaplasma* spp. (*U.parvum* and *U.urealyticum*) DNA amplification product.
– The channel for the ROX fluorophore (ROX channel or analogous, depending on the detector model) is intended for the detection of the signal of the *Mycoplasma genitalium* DNA amplification product.
– The channel for the Cy5 fluorophore (Cy5 channel or analogous, depending on the detector model) is intended for the detection of the signal of the IC DNA amplification product.

Before the detection run, the required settings of the detector software should be adjusted according to the *Important Product Information Bulletin* enclosed to the PCR kit and Guidelines [2].

The obtained results are interpreted on the basis of the level of fluorescence signal in the corresponding channels relatively to the background for the clinical and control samples. Interpretation is performed automatically by the software of the instrument used.

The principle of interpretation is the following:

— *Chlamydia trachomatis* DNA is **detected** if the signal determined in the FAM channel is greater than the specified threshold value of the positive result.
— *Ureaplasma* spp. (*U.parvum* and *U.urealyticum)* DNA is **detected** if the signal determined in the HEX channel is greater than the specified threshold value.
— *Mycoplasma genitalium* DNA is **detected** if the signal determined in the ROX channel is greater than the specified threshold value of the positive result.
— *Chlamydia trachomatis* DNA, *Ureaplasma* spp. DNA and *Mycoplasma genitalium* DNA are **not detected** if the signals determined in the FAM, HEX and ROX channel are less

than the specified threshold values of the negative result, whereas the signal determined in the Cy5 channel is greater than the specified threshold value.

- The result of the analysis is **invalid** if the signals of the sample determined in the FAM, HEX, ROX and Cy5 channels are less than the specified threshold values for these channels.
- The result of the analysis is **equivocal** if the signal of the sample determined in the FAM and/or ROX channel(s) is greater than the specified threshold value of the negative result but less than the threshold value of the positive result (the signal is between threshold values).

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

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

Table 2

Results for controls

Control	Stage for control	Signal in channel			
		FAM	HEX	ROX	Cy5
C–	DNA extraction	**<** threshold value of negative result	**<** threshold value	**<** threshold value of negative result	**>** threshold value
NCA	PCR	**<** threshold value of negative result	**<** threshold value	**<** threshold value of negative result	**<** threshold value
C+	PCR	**>** threshold value of positive result	**>** threshold value	**>** threshold value of positive result	**>** threshold value

10. TROUBLESHOOTING

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

1. If the signal determined for the Positive Control of Amplification (C+) in the FAM and/or HEX and/or ROX channels is less than the threshold value of the positive result, the amplification and detection should be repeated for all samples in which the signal in the FAM and/or HEX and/or ROX channels was less than the threshold value of the positive result.
2. If the signal determined for the Negative Control of Extraction (C–) and/or Negative Control of Amplification (NCA) in the FAM and/or HEX and/or ROX channels is greater than the threshold value of the positive result, the PCR analysis should be repeated for all samples in which the signal in the FAM and/or HEX and/or ROX channels was greater than the threshold value of the positive result.

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

11. TRANSPORTATION

AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-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.genitalium*-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.genitalium*-MULTIPRIME-FEP** PCR kit are stable until the expiration date stated 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 / M.genitalium* 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 genitalium	10^3
Urine[3]	DNA-sorb-AM	PCR kit variant FEP	*Chlamydia trachomatis*	10^3
			Ureaplasma spp.	$2x10^3$
			Mycoplasma genitalium	$2x10^3$

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

13.2. Specificity

The analytical specificity of the **AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FEP** PCR kit is ensured by the 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.

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

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

[3] Pretreatment is required.

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; Mycoplasma hominis; Ureaplasma urealyticum; Ureaplasma parvum; Mycoplasma genitalium; Chlamydia trachomatis; Neisseria* spp.*; Neisseria gonorrhoeae; Trichomonas vaginalis; Treponema pallidum; Toxoplasma gondii; HSV* types 1 and 2; *CMV; HPV.*

The clinical specificity of the **AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FEP** 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, 2010.
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”.

15. QUALITY CONTROL

In compliance with the Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-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”
15.07.15 ME	Text	Corrections according to the template
	1. Intended use	The clinical material was specified
	6. Sampling and handling	
	3. Content	For the PCR-mix-Background-red reagent, the note “is used if DNA samples were extracted using DNA-sorb-AM or DNA-sorb-B kits” was added