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For Professional Use Only

# **AmpliSens<sup>®</sup> *Treponema pallidum*-FEP**

PCR kit

**Instruction Manual**

## **AmpliSens<sup>®</sup>**



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<sup>®</sup> *Treponema pallidum*-FEP** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Treponema pallidum* DNA in clinical materials (urogenital, rectal, and oral swabs; blister exudate; and discharge of erosive-ulcer lesions of human skin and mucous membranes) 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

*Treponema pallidum* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *Treponema pallidum* primers. In **Fluorescent End-Point** 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 a reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens<sup>®</sup> *Treponema pallidum*-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<sup>®</sup> *Treponema pallidum*-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<sup>®</sup> *Treponema pallidum*-FEP** PCR kit is produced in 2 forms:

AmpliSens<sup>®</sup> *Treponema pallidum*-FEP PCR kit variant FEP (0.5-ml tubes),

**REF** B20-100-R0,5-FEP-CE.

AmpliSens<sup>®</sup> *Treponema pallidum*-FEP PCR kit variant FEP (0.2-ml tubes),

**REF** B20-100-R0,2-FEP-CE.

**AmpliSens<sup>®</sup> *Treponema pallidum*-FEP PCR kit includes:**

<b>Reagent</b>	<b>Description</b>	<b>Volume (ml)</b>	<b>Quantity</b>
<b>PCR-mix-1-FL <i>Treponema pallidum</i></b> ready-to-use single-dose test tubes ( <i>under wax</i> )	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>	red 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

\* used for thermocyclers without constant-temperature cover (for example, Terzik (DNA-Technology)).

\*\* used to analyze DNA samples extracted with DNA-sorb-AM extraction kit.

\*\*\* 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<sup>®</sup> *Treponema pallidum*-FEP PCR kit 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 tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 or GeneAmp PCR System 2400 (Applied Biosystems, USA), Uno-2 (Biometra, Germany), MiniCycler, PTC-100 (MJ Research, USA), MxyGene (Axygen, USA) or equivalent).

- Fluorometer (for example, ALA-1/4 (Biosan, Latvia) or equivalent).
- Personal computer.
- 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, 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 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 mucosa. If skin, eyes, or mucosa 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 are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

**AmpliSens® *Treponema pallidum*-FEP** PCR kit is intended for analysis of DNA extracted by using DNA extraction kits from urogenital, rectal, and oral swabs; blister exudate; and discharge of erosive-ulcer lesions of human skin and mucous membranes.

## 7. WORKING CONDITIONS

**AmpliSens® *Treponema pallidum*-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 kit:

- DNA-sorb-AM, **REF** K1-11-100-CE.



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 *Treponema pallidum*** 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 *Treponema pallidum***.
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**) if a thermocycler without constant-temperature cover is used.
4. Prepare one **Background** sample. To do this, mark one **PCR-mix-1-FL *Treponema pallidum*** tube as **Background** and add **20 µl** of **PCR-mix-Background-red** above the wax layer surface ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *Treponema pallidum***. Add above **1 drop** of **mineral oil for PCR** (if a thermocycler without a constant-temperature cover is used).



PCR-mix-Background-red is used if DNA was extracted using DNA-sorb-AM (**REF** K1-12-100-CE) or DNA-sorb-B (**REF** K1-2-100-CE). If any other nucleic acid extraction kit (recommended by CRIE) was used, follow the instructions provided by the manufacturer.

5. Add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage using tips with aerosol barrier.



The tubes with PCR-mix-1-FL *Treponema pallidum* that are not used at the moment should be stored away from light.

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).

### 8.2.2 Amplification

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

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

Table 1

**AmpliSens-1-FEP amplification program**

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



Program other thermocyclers according to Guidelines.

## 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 Guidelines.

Internal Control is detected in the HEX channel, *Treponema pallidum* DNA is detected in the FAM channel.

1. Principle of interpretation:

- *Treponema pallidum* DNA is **detected** in a sample if its signal in the FAM channel is greater than the defined threshold value of the positive result.
- *Treponema pallidum* DNA is **not detected** in a sample if the signal in the FAM channel is less than the defined threshold value of the negative result whereas the signal in the HEX channel is greater than the defined threshold value.
- The result is **invalid** in a sample if the signal in the FAM channel is less than the defined threshold value of the negative result whereas the signal in the HEX channel is less than the defined threshold value.
- 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 the analysis is 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).

Table 2

**Results for controls**

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel (samples)	HEX channel (IC)	
<b>C-</b>	DNA extraction	< threshold of negative result	> threshold	OK
<b>NCA</b>	Amplification	< threshold of negative result	< threshold	nd
<b>C+</b>	Amplification	> threshold of positive result	> threshold	OK

## 10. TROUBLESHOOTING

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

1. No positive signal in the positive control of amplification (C+) may indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of PCR, noncompliance of the storage conditions for kit components with the manufacturer's instruction, or the expiration of the reagent kit. Check programming of the thermocycler (see 8.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR once again for all samples.
2. If no signal was detected either in the channel for detection of the pathogen DNA or in the channel for detection of the Internal Control, the sample should be examined once again (PCR and detection). The same applies to the samples with equivocal results, because the fact that the specific signal does not exceed the threshold value is not



sufficient to consider a sample as positive. If equivocal results are obtained in the second run, the analysis should be repeated starting from the DNA extraction stage.

3. Positive signal in negative controls (C– and NCA) indicates reagent or sample contamination. In this case, the results of analysis must be considered as invalid. The analyses must be repeated and measures for detecting and eliminating the contamination source must be taken.

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

## 11. TRANSPORTATION

**AmpliSens® CMV-FRT** PCR kit should be transported at 2–8 °C for no longer than 5 days.

## 12. STABILITY AND STORAGE

All components of the **AmpliSens® Treponema pallidum-FEP** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® Treponema pallidum-FEP** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.



PCR-mix-1-FL *Treponema pallidum* is to be kept away from light.

## 13. SPECIFICATIONS

### 13.1. Sensitivity

Clinical material	Transport medium	Nucleic acid extraction kit	Sensitivity, GE/ml <sup>1</sup>
Urogenital swabs	Transport Medium for Swabs (REF 956-CE, REF 987-CE) or Transport Medium with Mucolytic (REF 952-CE, REF 953-CE)	DNA-sorb-AM	1x10 <sup>3</sup>

### 13.2. Specificity

The analytical specificity of **AmpliSens® Treponema pallidum-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 deposited in gene banks by sequence comparison analysis. The clinical specificity of **AmpliSens® Treponema pallidum-FEP** PCR kit was confirmed in laboratory clinical trials.

Nonspecific responses were absent while testing human DNA samples and DNA samples of the following microorganisms: *Gardnerella vaginalis*, *Lactobacillus* spp., *Escherichia*

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

*coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Candida albicans*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*; *Mycoplasma genitalium*, *Neisseria flava*, *Neisseria subflava*, *Neisseria sicca*, *Neisseria mucosa*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *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.

#### 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®** *Treponema pallidum*-FEP PCR kit has been tested against predetermined specifications to ensure consistent product quality.

## 16. KEY TO SYMBOLS USED

	Catalogue number		Sufficient for
	Batch code		Expiration Date
	<i>In vitro</i> diagnostic medical device		Consult instructions for use
	Version		Keep away from sunlight
	Temperature limitation	<b>NCA</b>	Negative control of amplification
	Manufacturer	<b>C-</b>	Negative control of extraction
	Date of manufacture	<b>C+</b>	Positive control of Amplification
	Authorised representative in the European Community	<b>IC</b>	Internal control
	Caution		

### List of Changes Made in the Instruction Manual

<b>VER</b>	<b>Location of changes</b>	<b>Essence of changes</b>
29.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"