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For Professional Use Only

# AmpliSens® *Chlamydia trachomatis*-EPh

## PCR kit

## Instruction Manual

**AmpliSens®**

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**TABLE OF CONTENTS**

## 1. INTENDED USE

**AmpliSens® *Chlamydia trachomatis*-EPh** PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Chlamydia trachomatis* DNA in the clinical material (urogenital and conjunctival swabs, urine sediment, prostate gland secretion, and synovial fluid) by using electrophoretic detection of the amplified products in agarose gel.

## 2. PRINCIPLE OF PCR DETECTION

*Chlamydia trachomatis* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using specific *Chlamydia trachomatis* primers. After PCR, the amplified product is detected in agarose gel. AmpliSens® *Chlamydia trachomatis*-EPh 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® *Chlamydia trachomatis*-EPh 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 the reaction mixture components mix only at 95 ºC.

## 3. CONTENT

**AmpliSens® *Chlamydia trachomatis*-EPh** PCR kit is produced in 2 forms:

AmpliSens® *Chlamydia trachomatis*-EPh PCR kit variant 100 R (0.5-ml tubes),

**REF** B1-100-R0,5-CE.

AmpliSens® *Chlamydia trachomatis*-EPh PCR kit variant 100 R (0.2-ml tubes),

**REF** B1-100-R0,2-CE.

AmpliSens® *Chlamydia trachomatis*-EPh PCR kit variant 100 R (0.2-ml tubes) in bulk[1],

**REF** B1-100-R0,2-CE-B.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

**AmpliSens® *Chlamydia trachomatis*-EPh** PCR kit variant 100 R includes:

| *Reagent* | *Description* | *Volume, ml* | *Quantity* |
|---|---|---|---|
| **PCR-mix-1-R *Chlamydia trachomatis*** ready-to-use single-dose test tubes (*under wax)* | colorless clear liquid | 0.005 | 110 tubes of 0.5 or 0.2 ml |
| **PCR-mix-2 blue** | blue clear liquid | 1.2 | 1 tube |
| **Mineral oil for PCR** | colorless viscous liquid | 4.0 | 1 vial |
| **Positive Control DNA *Chlamydia trachomatis* ($C_{+\ Chlamydia\ trachomatis}$)** | 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 complex ICc**** | colorless clear liquid | 1.0 | 1 tube |

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

** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-12-100-CE or DNA-sorb-B, **REF** K1-2-100-CE protocols).

AmpliSens® *Chlamydia trachomatis*-EPh PCR kit variant 100 R is intended for 110 reactions (including controls).

## 4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Agarose gel detection kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Vortex mixer.
- Desktop microcentrifuge with a rotor for 2-ml reaction tubes (RCF max. 16,000 *g*).
- PCR box or biological cabinet.
- Vacuum aspirator with a flask for removing supernatant.
- Tube racks.
- 1.5-ml sterile polypropylene tubes.
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature ≤ –16 °C.
- Waste bin for used tips.
- Permanent pen for labeling.

• Thermostat with controlled temperature and capable of incubating at 25°C and 100 °C.

• Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia) or MaxyGene (Axygen Scientific, USA)).

## 5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

• Use sterile pipette tips with aerosol filters 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 detection.

• When thawed, mix the components and centrifuge briefly.

• Use protective gloves, laboratory coats, 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 biological cabinet in compliance with appropriate biosafety practices.

• Clean and disinfect all sample or reagent spills with 0.5% sodium hypochlorite solutions or other suitable disinfectant.

• Avoid contact with the skin, eyes, and mucous membranes. If skin, eyes 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 Areas. Do not return samples, equipment, and reagents to the area where you carried out the previous step.

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 that this handbook is read before starting work.

AmpliSens® *Chlamydia trachomatis*-EPh PCR kit is intended for analysis of DNA extracted with DNA extraction kits from:

- Urogenital swabs;
- Urine sediment (use the first part of the stream);
- Prostate gland secretion;
- Synovial fluid;
- Conjunctival smears.

## 7. WORKING CONDITIONS

**AmpliSens® *Chlamydia trachomatis*-EPh** PCR kit should be used at 18–25 °C.

## 8. PROTOCOL

### 8.1. DNA extraction

It is recommended that the following nucleic acid extraction kits are used:

- DNA-sorb-AM, **REF** K1-12-100-CE.
- DNA-sorb-B (for the prostate gland secretion), **REF** K1-2-100-CE.

Extract DNA according to the manufacturer's protocol.

### 8.2. Preparing PCR

Total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

1. Take the required quantity of tubes with **PCR-mix-1-R *Chlamydia trachomatis*** and wax for amplification of DNA extracted from clinical and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with the reagents in the tube.
3. Add above 1 drop of **mineral oil for PCR** (~25 µl). This step can be omitted if a thermocycler with a constant-temperature cover is used.

### 8.2.2 Amplification

Use the prepared tubes for PCR. **Add 10 µl of DNA samples** obtained from clinical or control samples at the DNA extraction stage using tips with aerosol barrier above or under the oil layer.

Carry the **control amplification reactions:**

NCA -Add 10 µl of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).

C+ -Add 10 µl of **Positive Control DNA *Chlamydia trachomatis*** to the tube for Positive Control of Amplification.

Run the following program in the thermocycler (see Table 1). When the temperature reaches 95ºC (pause mode), insert tubes into the cells 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

Programming thermocyclers for *Chlamydia trachomatis* DNA amplification

| | Thermocyclers with active temperature adjustment: | | | | | | Thermocyclers with block temperature adjustment: | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Terzik (DNA-Technology)** | | | **GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research)** | | | **Uno II (Biometra), MiniCycler, PTC-100 (MJ Research)** | | |
| Step | 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** | 10 s | 42 | **95 °C** | 15 s | 42 | **95 °C** | 1 min | 42 |
| | **65 °C** | 10 s | | **65 °C** | 25 s | | **65 °C** | 1 min | |
| | **72 °C** | 10 s | | **72 °C** | 25 s | | **72 °C** | 1 min | |
| 3 | **72 °C** | 1 min | 1 | **72 °C** | 1 min | 1 | **72 °C** | 1 min | 1 |
| 4 | **4 °C** | storage | | **4 °C** | storage | | **10 °C** | storage | |

Amplification in thermocyclers with block and active temperature adjustment continues for 2 h 30 min and 1 h 50 min, respectively.

After the reaction is completed, PCR tubes should be collected and transferred to the room for PCR product analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2–8 °C, and for a long time at –16 °C (warm up samples to room temperature before electrophoretic run).

## 9. DATA ANALYSIS

It is recommended to use the following detection agarose kit:

- EPh variant 200, **REF** K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7%). The length of specific amplified DNA fragments is as follows:

- *Chlamydia trachomatis*, 330 bp;
- Internal Control, 740 bp.

Put on a protective mask or use a glass filter while visualizing and photographing the gel.

9.1. Interpretation of results

Table 2

**Results for controls**

| Control | Which step of test is controlled | Specific 330-bp bands in agarose gel | Specific 740-bp bands in agarose gel | Interpretation |
|---|---|---|---|---|
| C– | DNA extraction | No | Yes | OK |
| NCA | Amplification | No | No | OK |
| C+ | Amplification | Yes | No | OK |

- The sample is considered to be positive for *Chlamydia trachomatis* DNA if the 330-bp band is present in gel. The band of IC (740 bp) may be absent in samples with a high concentration of *Chlamydia trachomatis* DNA.
- The sample is considered to be negative for *Chlamydia trachomatis* DNA if the 330-bp band is absent whereas the 740-bp band is present in gel.

In addition to the specific bands, fuzzy bands corresponding to primer dimers may appear in lanes below the 100-bp level.

## 10. TROUBLESHOOTING

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

- If the results obtained for the control samples results do not correspond to the results for controls listed in Table 2, the appropriate stage of the test should be repeated. Discard any reagents that may be suspect.
- If neither the 330-kb nor the 740-kb band is detected in lanes, the result of analysis of this sample is irrelevant. In this case, analysis of this sample should be repeated starting from the DNA extraction stage. This may be caused by clinical processing errors that led to the loss of DNA or inhibition of PCR.
- The appearance of nonspecific bands of different molecular weight in lanes may be caused by the lack of “hot start” or an inappropriate temperature regime in the thermocycler. In this case, the results of analysis are invalid.
- The appearance of the specific 330-bp band in lanes corresponding to negative controls (NCA, C–) suggests contamination of reagents or samples. In such cases, the results of analysis are considered to be invalid. Analysis of all samples must be repeated and measures to detect and eliminate the source of contamination must be taken.

## 11. TRANSPORTATION

**AmpliSens® *Chlamydia trachomatis-EPh*** PCR kit should be transported at 2–8 ºC for no longer than 5 days.

## 12. STABILITY AND STORAGE

All components of the AmpliSens® *Chlamydia trachomatis*-EPh PCR kit are to be stored at 2–8 ºC when not in use. They 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.

## 13. SPECIFICATIONS

### 13.1. Sensitivity

The analytical sensitivity of AmpliSens® *Chlamydia trachomatis*-EPh PCR kit is not less than $5x10^3$ genome equivalents per 1 ml of sample (GE/ml).

The claimed analytical characteristics of AmpliSens® *Chlamydia trachomatis*-EPh PCR kit are guaranteed only when additional reagent kits DNA-sorb-AM or DNA-sorb-B (for the prostate gland secret) and EPh, manufactured by FBIS CRIE, are used.

### 13.2. Specificity

The specificity of AmpliSens® *Chlamydia trachomatis*-EPh PCR kit is ensured by selection of specific primers and stringent reaction conditions. It was confirmed in laboratory and clinical trials.

## 14. REFERENCES


1. Manual “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”, Moscow, 2008.


## 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® *Chlamydia trachomatis*-EPh PCR kit is tested against predetermined specifications to ensure consistent product quality.

## 16. KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number |  | Caution |
| LOT | Batch code |  | Sufficient for |
| RUO | Research use only |  | Expiration Date |
| VER | Version |  | Consult instructions for use |
|  | Temperature limitation | **NCA** | Negative control of amplification |
|  | Manufacturer | **C–** | Negative control of extraction |
|  | Date of manufacture | **C+** | Positive control of amplification |
| **FBIS CRIE** | Federal Budget Institute of Science "Central Research Institute for Epidemiology" | **ICc** | Internal control complex |

**List of Changes Made in the Instruction Manual**

| VER | Location of changes | Essence of changes |
|---|---|---|
| 29.06.11 VV | Cover page, text | The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology” |
| | Cover page | The phrase “For Professional Use Only” was added |
| | Content | The “Explanation of Symbols” section was renamed to “Key to Symbols Used” |
| | Key to Symbols Used | The explanation of symbols was corrected |
| 12.07.11 VV | Content | New sections “Working conditions “and “Transportation” were added |
| | Stability and Storage | The information about the shelf life of open reagents was added |
| 11.04.12 Ivl | Title page, Key to symbols used | Symbol IVD *in vitro* diagnostic medical device was changed to RUO research use only |
| 14.10.13 ME | Content | The form in bulk was added |
| | Footer | **REF** B1-100-R0,2-CE-B was added |