CE 1023

IVD

For Professional Use Only

AmpliSens® *Chlamydia trachomatis*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® ***Chlamydia trachomatis*-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Chlamydia trachomatis* DNA in the clinical materials (urogenital, rectal, and throat swabs; eye discharge; urine; and prostate gland secretion) by means of real-time hybridization-fluorescence detection.

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Chlamydia trachomatis detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific primers. In real-time PCR the amplified product is detected by using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens**® ***Chlamydia trachomatis*-FRT** PCR kit is a qualitative test that contains the Internal Control (IC), which 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*-FRT** 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 or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 ºC. Chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min.

3. CONTENT

AmpliSens® ***Chlamydia trachomatis*-FRT PCR kit** is produced in 3 forms:

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit variant FRT (for use with RG),

REF R-B1(RG)-CE.

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit variant FRT (for use with iQ),

REF R-B1(iQ)-CE.

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit variant FRT-100 F

REF R-B1-F(RG,iQ)-CE.

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit, variant FRT includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
PCR-mix-1-FL *Chlamydia trachomatis* ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.01	110 tubes of 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	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 in the extraction procedure as Negative Control of Extraction.

** add 10 µl of Internal Control-FL during the DNA extraction procedure directly to the sample/lysis mixture (see "DNA-sorb-AM" **REF** K1-12-100-CE protocol).

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit is intended for 110 reactions, including controls.

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit, variant FRT-100 F includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
PCR-mix-1-FL *Chlamydia trachomatis*	colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
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 in the extraction procedure as Negative Control of Extraction.

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

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.

- Transport medium.
- Disposable powder-free gloves.
- 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.
- Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia) Instrument; iCycler iQ or iQ5 (Bio-Rad, USA) Instrument.
- Disposable polypropylene microtubes for PCR (0.1- 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 extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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 specimens and unused reagents in accordance with local authorities' regulations.
- Specimens 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 other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.

- 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

Sampling of biological materials for PCR-analysis, transportation, and storage are described in detail in handbook of the manufacture [1]. It is recommended that this handbook is read before beginning of the work.

AmpliSens® *Chlamydia trachomatis*-FRT PCR kit is intended to analyze DNA extracted with DNA extraction kits from:

— *urogenital, rectal, and throat swabs;*

— *eye discharge;*

— *urine (the sediment of the first portion of morning urine);*

— *prostate gland secretion.*

7. WORKING CONDITIONS

AmpliSens® *Chlamydia trachomatis*-FRT 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.
- Other nucleic acid extraction kits recommended by CRIE.

Please extract DNA according to the instructions provided by the manufacturer.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

Variant FRT

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

1. Collect the required number of the tubes with **PCR-mix-1-FL *Chlamydia trachomatis*** 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 wax layer of each tube, so that it does not fall under the wax and mix with **PCR-mix-1-FL *Chlamydia trachomatis.***

Variant FRT-100 F

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

1. Thaw the **PCR-mix-2-FRT** tube. Vortex the tubes with **PCR-mix-1-FL *Chamydia trachomatis*, PCR-mix-2-FRT, and polymerase (TaqF)** and then centrifuge briefly.
   Take the required number of strip or unstrip tubes for amplification of DNA from clinical and control samples (0.2-ml tubes for a 36-well rotor or 0.1-ml strip tubes for a 72-well rotor).
2. For N reactions (including 2 controls), add to a new tube:
   10*(N+1) µl of **PCR-mix-1-FL *Chlamydia trachomatis***,
   5.0*(N+1) µl of **PCR-mix-2-FRT,**
   0.5*(N+1) µl of **polymerase (TaqF).**
   Vortex the tube and then centrifuge briefly. Transfer **15 µl** of the prepared mixture to each tube.

Steps 3 and 4 are applied for both variants.

3. Using tips with aerosol barrier, add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction to the prepared tubes.
4. Perform 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).

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

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines **"Real-Time PCR Detection of STIs and Other Reproductive Tract Infections**" [2].

1. Create a temperature profile on your instrument as follows:

AmpliSens-1 program

	Rotor-type Instruments[1]			Plate-type Instruments[2]		
Step	***Temperature, °C***	***Time***	**Repeats**	***Temperature, °C***	***Time***	**Repeats**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s *fluorescent signal detection*		**60**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in FAM/Green and JOE/Yellow/Hex fluorescence channels.

2. Adjust the fluorescence channel sensitivity according to **Important Product Information Bulletin***.*

9. DATA ANALYSIS

The fluorescent signal intensity is detected in two channels:

– The signal from the *Chlamydia trachomatis* DNA amplification product is detected in the FAM/Green channel;

– The signal from the Internal Control amplification product is detected in the JOE/Yellow/HEX channel.

Interpretation of results

The results are interpreted by the software of the instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

Principle of interpretation:

— *Chlamydia trachomatis* DNA is **detected** in a sample if its Ct value is present in the FAM channel. The fluorescence curve should cross the threshold line in the area of exponential fluorescence growth.

— *Chlamydia trachomatis* DNA is **not detected** in a sample if its Ct value is absent in the FAM channel (fluorescence curve does not cross the threshold line) while the Ct value in the JOE channel is less than the specified boundary value.

— The result is **invalid** if the Ct value of a sample in the FAM channel is absent while the Ct value in the JOE channel is either absent or greater than the specified boundary value. It is necessary to repeat the PCR analysis of such samples.

[1] For example,Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[2] For example, iCycler, iQ5, Mx3000P, Mx3000, “DT-96” or equivalent.

Ct boundary values are specified in the *Important product information bulletin* enclosed in the PCR kit.
Also refer to the Guidelines “Real-Time PCR Detection of STIs and Other Reproductive Tract Infections.”

The result of analysis is considered reliable only if the results obtained for 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	Ct value in channel		Interpretation
		FAM	JOE	
C–	DNA extraction	Neg	Pos (< boundary value)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< boundary value)	Pos (< boundary value)	OK

10. TROUBLESHOOTING

- If the Ct value of the Positive Control of Amplification (C+) in the FAM channel is absent or greater than the boundary Ct value, repeat PCR analysis of all samples in which *Chlamydia trachomatis* DNA was not found.
- If a Ct value is detected for the Negative Control of Extraction (C–) and/or Negative Control of Amplification (NCA) in the FAM channel, repeat PCR analysis for all samples in which *Chlamydia trachomatis* DNA was found starting from the DNA extraction stage.

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

11. TRANSPORTATION

AmpliSens® *Chlamydia trachomatis*-FRT 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*-FRT** PCR kit (except for Polymerase (TaqF) and PCR-mix-2-FRT) 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.

Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 ºC when not in use.

PCR-mix-1-FL *Chlamydia trachomatis* should be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Chlamydia trachomatis*-FRT** PCR kit is specified in the table below.

Clinical material	Transport medium	DNA extraction kit	Analytical sensitivity, GE/ml*
Urogenital swabs	Transport Medium for Swabs or Transport Medium with Mucolytic	DNA-sorb-AM	5×10^2
Urine (pretreatment is required)	–	DNA-sorb-AM	1×10^3

* Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

13.2. Specificity

The analytical specificity of **AmpliSens® *Chlamydia trachomatis*-FRT** PCR kit is ensured by selection of specific primers and probes as well as by selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. There were no nonspecific responses during examination of human DNA as well as DNA panel of the following microorganisms: *Gardnerella vaginalis, Lactobacillus spp., Escherichia 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, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* type 1 and 2, *CMV,* and *HPV.*

The clinical specificity of **AmpliSens® *Chlamydia trachomatis*-FRT** 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, 2008.
2. Guidelines “Real-Time 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®** ***Chlamydia trachomatis*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”