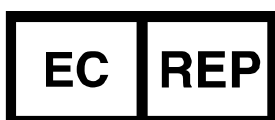




For Professional Use Only

**AmpliSens[®] *C.trachomatis* / *Ureaplasma* /
M.genitalium / *M.hominis*-MULTIPRIME-FRT
PCR kit
Instruction Manual**

AmpliSens[®]



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1. INTENDED USE

AmpliSens® C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-FRT 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*), *Mycoplasma genitalium* and *Mycoplasma hominis* in the clinical material (urogenital, rectal and oropharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) using real-time 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* / *Mycoplasma genitalium* / *Mycoplasma hominis* detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific regions using specific *Chlamydia trachomatis* / *Ureaplasma* / *Mycoplasma genitalium* / *Mycoplasma hominis* primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. 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® C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-FRT 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 / M.hominis-MULTIPRIME-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 °C. Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

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

AmpliSens® C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-FRT PCR kit variant FRT, **REF** R-B60(RG)-CE.

AmpliSens® C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-FRT PCR kit variant FRT-100 F, [REF] R-B60-F(RG)-CE.

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

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL C.trachomatis / Ureaplasma / M.genitalium / M.hominis (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 (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 / M.hominis-MULTIPRIME-FRT PCR kit variant FRT is intended for 110 reactions, including controls.

AmpliSens® C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-FRT PCR kit variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL C.trachomatis / Ureaplasma / M.genitalium / M.hominis	colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	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® *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*-MULTIPRIME-FRT PCR kit variant FRT-100 F is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
 - a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
 - b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at 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 all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance 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 in 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 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.genitalium* / *M.hominis*-MULTIPRIME-FRT PCR kit is intended for analysis of DNA extracted with DNA extraction kits from the clinical material (urogenital swabs, rectal swabs, oropharyngeal swabs, conjunctival discharge and prostate gland secretion, urine samples).

7. WORKING CONDITIONS

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

- 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 STI-FL (IC)**.



Extract DNA according to the manufacturer's protocol.

8.2. Preparing PCR

8.2.1. Preparing tubes PCR

Variant FRT

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

1. Prepare the required number of the tubes with **PCR-mix-1-FL *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*** 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* / *M.hominis***.

Variant FRT-100 F

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

1. Take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples.
2. For N reactions, add to a new tube:
 - 10*(N+1) µl of PCR-mix-1-FL *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*,**
 - 5.0*(N+1) µl of PCR-mix-2-FRT,**
 - 0.5*(N+1) µl of polymerase (TaqF).**

Vortex the tube, then centrifuge shortly. Transfer **15 µl** of the prepared mix to each tube.

Steps 3 and 4 are required in both variants.

3. Using tips with aerosol filter, add **10 µl** of **DNA** obtained at the DNA extraction stage.
4. 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).

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

8.2.2. Amplification

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

AmpliSens-1 amplification program

Step	Temperature, °C	Time	Cycles
Hold	95	15 min	1
Cycling	95	5 s	5
	60	20 s	
	72	15 s	
Cycling 2	95	5 s	40
	60	20 s (fluorescence detection)	
	72	15 s	

Fluorescent signal is detected in the channels for the FAM, JOE, ROX, Cy5 and Cy5.5 fluorophores.

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in five channels:

- The signal of the ***Chlamydia trachomatis* DNA** amplification product is detected in the channel for the FAM fluorophore.
- The signal of the ***Ureaplasma spp.* DNA** amplification product is detected in the channel for the JOE fluorophore,
- The signal of the ***Mycoplasma genitalium* DNA** amplification product is detected in the channel for the ROX fluorophore,
- The signal of the ***Mycoplasma hominis* DNA** amplification product is detected in the channel for the Cy5.5 fluorophore,
- The signal of the **Internal Control DNA** amplification product is detected in the channel for the Cy5 fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

1. ***Chlamydia trachomatis* DNA is detected** if the *Ct* value is determined in the results grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of

fluorescence.

2. ***Ureaplasma* spp. (*U.parvum* and *U.urealyticum*) DNA is detected** if the *Ct* value is determined in the results grid in the channel for the JOE fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
3. ***Mycoplasma genitalium* DNA is detected** if the *Ct* value is determined in the results grid in the channel for the ROX fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
4. ***Mycoplasma hominis* DNA is detected** if the *Ct* value is determined in the results grid in the channel for the Cy5.5 fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
5. ***Chlamydia trachomatis*, *Ureaplasma* spp. (*U.parvum* and *U.urealyticum*), *Mycoplasma genitalium*, and *Mycoplasma hominis* DNA are not detected** in a sample if the *Ct* value is not determined (absent) (fluorescence curve does not cross the threshold line) in the channels for FAM, JOE, ROX and Cy5.5 fluorophores whereas the *Ct* value determined in the channel for the Cy5 fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*.
6. The result is **invalid** if the *Ct* value is not determined (absent) in the channel for Cy5 fluorophore or greater than the specified boundary *Ct* value, whereas the *Ct* value in the channel for the FAM, JOE, ROX and Cy5.5 fluorophores is not determined (absent) or greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated starting from the DNA extraction stage



Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The result of the 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 (see Table 2).

Table 2

Results for controls

Control	Stage for control	Ct value in the channel for fluorophore	
		FAM, FOE, ROX and Cy5.5	Cy5
C-	DNA extraction	Absent	<boundary value
NCA	PCR	Absent	Absent
C+	PCR	<boundary value	<boundary value

10. TROUBLESHOOTING

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

1. If no signal is detected for Positive Control of Amplification (C+) or the signal is greater than the specified boundary *Ct* value in the channels for the **FAM, ROX, JOE** and **Cy5.5** fluorophores, PCR analysis should be repeated starting from the extraction stage for all samples for which *Ct* values in these channels were not detected.
2. If a *Ct* value is determined for the Negative Control of Extraction (C-) and/or for the Negative Control of Amplification (NCA) in the channels for the **FAM, ROX, JOE** and **Cy5.5** fluorophores, PCR analysis should be repeated for all samples for which a *Ct* value in these channels was determined.
3. If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample with a fluorescence curve without the area of typical exponential growth (the fluorescence curve is approximately linear), this may indicate incorrect setting of the threshold line or incorrect calculation parameters of baseline. Such a result should not be considered as positive. If such result was obtained in the presence of the correct setting of threshold line, PCR analysis of the sample should be repeated.

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.genitalium / M.hominis-MULTIPRIME-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® C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for polymerase (TaqF) and PCR-mix-2-FRT). All components of the **AmpliSens® C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-FRT** PCR kit are stable until the expiry date stated 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 the temperature from minus 24 to minus 16 °C when not in use.



PCR-mix-1-FL *C.trachomatis / Ureaplasma / M.genitalium / M.hominis* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity for *Chlamydia trachomatis*, *Ureaplasma* spp., *Mycoplasma genitalium*, and *Mycoplasma hominis* is not less than 5×10^2 genome equivalents per 1 ml of sample (GE/ml).

The analytical sensitivity for each microorganism is preserved in the presence of high DNA concentrations of other analyte microorganism (for example, in case of mixed-infections).

13.2. Specificity

The analytical specificity of **AmpliSens[®] C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The clinical specificity of **AmpliSens[®] C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-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, 2010.
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[®] C.trachomatis / Ureaplasma / M.genitalium / M.hominis-MULTIPRIME-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	<i>In vitro</i> diagnostic medical device		Expiration Date
	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
	Authorised representative in the European Community	C+	Positive control of amplification
		IC	Internal control

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"
23.07.15 PM	Through the text	Corrections in accordance with the template
	1. Intended use	The clinical material was specified
	6. Sampling and handling	
	3. Content	The volume of PCR-mix-1-FL <i>C.trachomatis</i> / <i>Ureaplasma</i> / <i>M.genitalium</i> / <i>M.hominis</i> for PCR kit variant FRT-100 F was changed from 1.1 to 1.2 ml
	9. Data analysis	The sections were rewritten
10. Troubleshooting		