



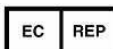
For *In Vitro* Diagnostic Use

AmpliSens® *Mycoplasma genitalium*-FRT

PCR kit

Instruction Manual

AmpliSens®



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1. INTENDED USE.

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycoplasma genitalium* DNA in the clinical materials (cervical, urethral scrapes (swabs), urine sediment, secrete of the prostate gland) by using real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION.

Mycoplasma genitalium detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Mycoplasma genitalium* primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. AmpliSens® *Mycoplasma genitalium*-FRT PCR kit is a qualitative test, which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. AmpliSens® *Mycoplasma genitalium*-FRT PCR kit uses "hot-start", which greatly reduces frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer or chemically modified polymerase (TaqF). Wax melting and reaction mix components occur only at 95 °C. Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT.

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit is produced in 3 forms:

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit variant FRT (for use with RG) [REF](#) R-B4(RG)-CE.

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit variant FRT (for use with iQ) [REF](#) R-B4(iQ)-CE.

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit variant FRT-100 F (for use with RG, iQ) [REF](#) R-B4-F(RG, iQ)-CE.

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit, variant FRT includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT <i>Mycoplasma genitalium</i> ready-to-use single-dose test tubes (under wax)	colorless, clear liquid	0.008	110 tubes
PCR-mix-2-FL	colorless, clear liquid	0.77	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 isolation procedure as Negative Control of Extraction.

** add 10 µl of Internal Control-FL during the DNA isolation procedure directly to the sample/lysis mixture (see "DNA-sorb-AM" [REF](#) K1-12-

100-CE, "DNA-sorb-B",

[REF](#) K1-2-100-CE protocols).

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

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit, variant FRT-100 F includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT <i>Mycoplasma genitalium</i>	colorless, clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless, clear liquid	0.3	2 tubes
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 isolation procedure as Negative Control of Extraction.

** add 10 µl of Internal Control-FL during the DNA isolation procedure directly to the sample/lysis mixture (see “DNA-sorb-AM” [REF](#) K1-12-100-CE, “DNA-sorb-B”, [REF](#) K1-2-100-CE protocols).

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

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit
- 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 rotor for 2 ml reaction tubes
- PCR box
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia) Instrument; iQ5 or iQ iCycler (BioRad, USA) Instrument
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator for 2–8 °C
- Deep-freezer with temperature not more than minus16°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 detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable 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 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 contact with the skin, eyes and mucosa. If skin, eyes and mucosa 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 move to the Amplification and Detection Area. 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 is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit is intended for the analysis of DNA extracted with DNA isolation kits from cervical or urethral scrapes (swabs), urine sediment (use the first portion of the morning specimen), or secrete of the prostate gland.

7. PROTOCOL.

7.1. DNA Isolation

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

- “DNA-sorb-AM”, [REF](#) K1-12-100-CE.
- “DNA-sorb-B”, [REF](#) K1-2-100-CE (for secret of the prostate gland).



Carry out the DNA isolation according to the manufacturer's instructions.

7.2. Preparing the PCR.

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

7.2.1 Preparing tubes for PCR.

Variant FRT

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *Mycoplasma genitalium*** and wax for amplification of DNA from clinical and control samples.
2. Add 7 µl of **PCR-mix-2-FL** 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-FEP/FRT *Mycoplasma genitalium***.

Variant FRT-100F

1. Prepare the required number of the tubes for amplification of DNA from clinical and control samples (0.2 ml tubes for 36-Well rotor or 0.1 ml stripes for 72-Well rotor).
2. For carrying of N reactions (including 2 controls) mix in a new tube: **10*(N+1) µl of PCR-mix-1-FEP/FRT *Mycoplasma genitalium*, 5.0*(N+1) µl of PCR-mix-2-FRT and 0.5*(N+1) µl of polymerase (TaqF)**. Vortex the tube, then centrifuge shortly. Transfer 15 µl of prepared mix into 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 DNA extraction stage into prepared tubes.



The tubes with PCR-mix-1-FEP/FRT *Mycoplasma genitalium* are to be kept away from light when not in use

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).

7.2. 2. Amplification

7.2.2.1. RG

1. Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
2. Create a temperature profile on your Rotor-Gene™ instrument as follows:

AmpliSens-1 RG program

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling 2	95	5 sec	–	40
	60	20 sec	FAM/Green, JOE/Yellow	
	72	15 sec	–	



AmpliSens-1 RG general program allows simultaneous conducting of any combination of tests for detection of sexually transmitted diseases pathogens DNA including tests for identifying of *Human Papillomaviruses* by means of AmpliSens HPV HCR PCR kits.



If “multiprime” format tests for detection of sexually transmitted diseases (AmpliSens PCR kits) are carried out simultaneously, the program and template corresponding to those “multiprime” tests should be applied.

3. Fluorescence detection is on the 2-nd pass (**60°C**) in FAM/Green and JOE/Yellow fluorometer channels.
4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

7.2.2.2. iQ

1. Program the iQ™ according to manufacturer's manual and Appendix 2.

2. Create a temperature profile on your iQ™ instrument as follows:

AmpliSens-1 iQ program				
Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling 2	95	5 sec	–	40
	60	30 sec	FAM-490, HEX-530	
	72	15 sec	–	



AmpliSens-1 iQ general program allows a combination of test for the detection of pathogens of sexually transmitted disease to be carried out simultaneously, including tests for the identification of *Human Papillomaviruses* using AmpliSens HPV HCR PCR kits.

- Fluorescence detection is on the 2-nd pass (60°C) in FAM and HEX fluorometer channels.
- Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.

8. DATA ANALYSIS.

RG. Internal Control is detected on the JOE /Yellow fluorescence channel, *Mycoplasma genitalium* DNA is detected on the FAM /Green fluorescence channel.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

iQ. Internal Control is detected on the HEX fluorescence channel, *Mycoplasma genitalium* DNA is detected on the FAM fluorescence channel.

See **Appendix 2** for data analysis settings for iQ5 or iQiCycler.

Results interpretation

The results are interpreted by the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 or iQ5 or iQiCycler Instrument by the crossing (or not) of the fluorescence curve with the threshold line.

Results for controls

Control	Stage for control	Ct channel FAM/Green / FAM	Ct channel JOE/Yellow / HEX	Interpretation
C-	DNA isolation	Neg	Pos (< X*)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< Y*)	Neg	OK

*For X, Y values see Appendix 1 in case of using Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument or Appendix 2 in case of using iQ5 or iQiCycler Instrument.

- The sample is considered to be positive for *Mycoplasma genitalium* if its Ct value is defined in the results grid in FAM/Green channel.
- The sample is considered to be negative for *Mycoplasma genitalium* if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM/Green channel and in the results grid in the JOE/Yellow / HEX channel the Ct value doesn't exceed X.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

9. TROUBLESHOOTING.

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

- If Ct value is absent on both JOE/Yellow / HEX and FAM/Green channels or the Ct value in JOE/Yellow / HEX channel is higher than X, PCR reaction should be repeated. If the same result is achieved, the sample extraction process should be repeated. If the IC signal of this sample was detected normally during any other PCR test, then the second examination of the extraction stage should be omitted

(in case of using iQ5 or iQiCycler Instrument).

- If the Ct value is present for the Negative Control of extraction (C-) on FAM/Green channel and/or for Negative Control of Amplification on FAM, JOE/Yellow / HEX channels in the results grid, it indicates the contamination of reagent or samples. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures to detect and eliminate the source of contamination are to be taken.
- If no signal is detected for Positive Controls of amplification, it can suggest incorrect programming of the temperature profile of used Instrument, incorrect configuration of the PCR reaction or storage conditions for kit components has not complied with manufacturer instruction, or the reagents kit has expired. Programming of the used Instrument, storage conditions, and the expiration date of the reagents should be checked, and then the PCR should be repeated
- If a positive result (fluorescence curve crosses the threshold line) is registered for the sample that has a fluorescence curve without a typical exponential growth (the graph is linear). This can suggest incorrect setting of the threshold line or incorrect calculation of base line parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, the PCR should be repeated for the sample (in case of using iQ5 or iQiCycler Instrument).

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens® Mycoplasma genitalium-FRT** PCR kit (except for Polymerase(TaqF) and PCR-mix-2-FRT) are to be stored at between 2°C and 8°C, when not in use. All components of the **AmpliSens® Mycoplasma genitalium-FRT** PCR kit are to be stable until labeled expiration date.



Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at not more than minus 16°C

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® Mycoplasma genitalium FRT** PCR kit is no less than 1x10³ genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens® Mycoplasma genitalium-FRT** PCR kit are guaranteed only when additional reagents kits "DNA-sorb-AM" or "DNA-sorb-B"(manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

11.2. Specificity.

Specificity of **AmpliSens® Mycoplasma genitalium-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® Mycoplasma genitalium-FRT** PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES.

- Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL.

In compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Quality Management System, each lot of **AmpliSens® Mycoplasma genitalium-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQ.iCycler



Negative control