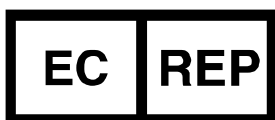




For Professional Use Only

AmpliSens[®] *M.genitalium*-screen-titre-FRT
PCR kit
Instruction Manual

AmpliSens[®]



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1. INTENDED USE

AmpliSens[®] *M.genitalium*-screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Mycoplasma genitalium* DNA in clinical materials (urogenital swabs taken from cervix, vagina or urethra as well as urine samples) by using real-time hybridization-fluorescence detection.



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

2. PRINCIPLE OF PCR DETECTION

Clinical material is taken and placed in a transport medium for storage and transportation of clinical samples. DNA is extracted from the clinical samples and used for PCR with hybridization fluorescent detection (real-time PCR).

Quantitative detection of DNA by real-time PCR is based on the linear dependence between the initial concentration of target DNA in a test sample and the cycle when the fluorescent signal begins to increase exponentially (cycle threshold, Ct). For quantitative detection, DNA of clinical samples is amplified simultaneously with DNA standards (samples with a known concentration of target DNA). The results of amplification of DNA standards are used for construction of a calibration curve and calculation of the target DNA concentration in test samples.

In the **AmpliSens[®] *M.genitalium*-screen-titre-FRT** PCR kit, the concentration of *M.genitalium* DNA can be determined in two variants. In the first variant, the number of genome equivalents of microorganism cells per 1 ml of clinical sample (GE/ml) is determined. Thus obtained values reflect the absolute concentration of microorganisms in the clinical material. In the second variant, the ratio between *M.genitalium* genomes and genomes of human mucosa cells is calculated. In this case, PCR mix contains not only the primers and probes for *M.genitalium* DNA but also the primers and probes for the human β -globin gene fragment. DNA standard solutions contain *M.genitalium* DNA standards as well as human DNA standards. Thus obtained relative values of *M.genitalium* DNA and human DNA concentrations reflect the density of the microorganisms on the mucosa. In addition, human DNA serves as an endogenous internal control which helps to monitor the quality of clinical material sampling.

Mycoplasma genitalium detection is based on the amplification of the pathogen genome specific region using specific *Mycoplasma genitalium* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that 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® *M.genitalium*-screen-titre-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 chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *M.genitalium*-screen-titre-FRT PCR kit is produced in 1 form:

AmpliSens® *M.genitalium*-screen-titre-FRT PCR kit variant FRT-100 F (for use with RG, iQ, Mx) **REF** R-B4-100-FT(RG,iQ,Mx)-CE.

AmpliSens® *M.genitalium*-screen-titre-FRT PCR kit variant FRT-100 F includes:

<i>Reagent</i>		<i>Description</i>	<i>Volume, ml</i>	<i>Quantity</i>
PCR-mix-1-FL <i>M.genitalium</i>-screen-titre		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
DNA-buffer		colorless clear liquid	0.5	1 tube
DNA calibrators	UG1	colorless clear liquid	0.1	1 tube
	UG2	colorless clear liquid	0.1	1 tube
Negative Control (C-)*		colorless clear liquid	1.2	1 tube

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

AmpliSens® *M.genitalium*-screen-titre-FRT 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 rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett

Research, Australia); Rotor-Gene Q (Qiagen, Germany), iCycler iQ or iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).

- 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 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, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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, and 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 DNA amplification techniques.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then 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 the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *M.genitalium*-screen-titre-FRT PCR kit is intended for the analysis of DNA extracted by DNA extraction kits from:

- urogenital swabs placed in a transport medium (manufactured or recommended by FBIS CRIE);
- urine (first portion).

7. WORKING CONDITIONS

AmpliSens® *M.genitalium*-screen-titre-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.



Extract DNA according to the manufacturer's instructions.



Extraction of DNA by EDEM reagent kit or any other express methods are not recommended.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

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

Prepare the reaction mixture straight before the test. Mix reagents for one reaction in the following proportion:

- **10 µl of PCR-mix-1-FL *M.genitalium*-screen-titre,**
- **5 µl of PCR-mix-2-FRT and polymerase (TaqF) mixture.**

1. Before starting work, it is necessary to prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. Transfer the content of one tube with **polymerase (TaqF) (30 µl)** to the tube with **PCR-mix-2-FRT (300 µl)** avoiding foaming. Mark each tube with the mixture preparation date.



The prepared mixture is intended for analysis of 60 samples. The mixture should be stored at 2–8 °C until use (not longer than for 3 months).



If the mixture cannot be utilized within 3 months, it should be prepared for a smaller number of reactions. For example, mix **150 µl of PCR-mix-2-FRT** and **15 µl of polymerase (TaqF)**. Thus prepared mixture is intended for 30 reactions.

2. Thaw and vortex the tube with **PCR-mix-1-FL *M.genitalium*-screen-titre**. Centrifuge shortly to remove the drops from the caps of the tubes.

Calculate the required number of reactions including the test and control samples according to Appendix 1. Note that even for analysis of one test DNA sample, it is necessary to run 4 controls: 2 calibrators (UG1 and UG2), the Negative Control of Amplification (DNA-buffer), and Negative Control of Extraction (Negative Control, C-).

It is necessary to take reagents for one extra reaction: for N tests, prepare reagents for (N+1) reactions.

3. Prepare the reaction mixture in an individual tube. Mix **PCR-mix-1-FL *M.genitalium*-screen-titre and PCR-mix-2-FRT, and polymerase (TaqF)**, which was prepared as described in item 1 of Section 8.2.1.
4. Prepare the required number of tubes for amplification of DNA from clinical and control samples.
5. Transfer **15 µl** of prepared reaction mixture into the tubes.
6. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage to the prepared tubes using tips with aerosol barrier.
7. Carry out the control reactions:

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

Calibrators UG1 and UG2 - Add **10 µl** of **UG1** and **10 µl** of **UG2** to the two tubes.

C- - Add **10 µl** of sample extracted from **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 [2].

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

AmpliSens-1 amplification program

Step	Rotor-type instruments ¹			Plate-type instruments ²		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
Hold	95	15 min	1	95	15 min	1
Cycling 1	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
Cycling 2	95	5 s	40	95	5 s	40
	60	20 s <i>fluorescent signal detection</i>		60	30 s <i>fluorescent signal detection</i>	
	72	15 s		72	15 s	

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

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

9. DATA ANALYSIS

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

- ***Mycoplasma genitalium* DNA** is detected in the **FAM/Green** fluorescence channel,
- **IC (endogenous internal control) DNA** is detected in the **JOE/Yellow** fluorescence channel.

See the **Manufacturer's manual, Guidelines** and **Important product information bulletin** for data analysis settings.

Interpretation of results

The results are interpreted by the crossing (or not crossing) of the fluorescence curve with the threshold line set at a level of exponential fluorescence growth, which determines the presence (or absence) of Ct values for this sample in corresponding cell in the results table. A calibration curve is constructed and the concentrations of *M.genitalium* and human DNA are calculated on the basis of the obtained Ct values of DNA standards.



Ct values for DNA standards (calibrators) are specified in the Important Product Information Bulletin

The final concentration of *M.genitalium* DNA can be expressed as absolute and relative

¹ For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.

² For example, iCycler, iQ5, Mx3000P, Mx3000 or equivalent.

(normalized) value.

Absolute *M.genitalium* concentration

The absolute concentration of *M.genitalium* DNA indicates the total content of this microorganism in the clinical sample placed in a transport medium. On the basis of the calibrator values specified, the instrument software automatically calculates the initial number of *M.genitalium* DNA copies in the reaction mixture and displays it in the result grid. The obtained data are used to calculate the number of *M.genitalium* genome equivalent in 1 ml of clinical sample.

$$\text{[Number of copies] } M.genitalium \text{ DNA} \times 200 = \text{[Number of genome equivalents]} \\ M.genitalium \text{ in 1 ml (GE/ml)}$$

Relative (normalized) *M.genitalium* concentration

The normalized concentration of *M.genitalium* indicates the number of cells of the pathogen relative to the number of mucous cells. In addition, human DNA concentration reflects material sampling quality. On the basis of the specified values of calibrators of *M.genitalium* and human DNA, the instrument software automatically calculates the initial number of *M.genitalium* DNA copies as well as the number of human DNA copies in a reaction and displays it in the result table. The obtained *M.genitalium* genome equivalents are normalized for 100,000 human cells by the following formula:

$$\frac{\text{[Number of copies] } M.genitalium \text{ DNA}}{\text{[Number of copies] human DNA}} \times 200,000 = \text{[Number of GE] } M.genitalium \text{ in } 10^5 \text{ human cells}$$



For details, see the interpretation of results in the Guidelines [2].

10. TROUBLESHOOTING

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

- If the Ct value is absent in both JOE/Yellow and FAM/Green channels or the Ct value in the JOE/Yellow /HEX channel is higher than the specified boundary value. PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (if iCycler iQ or iQ5 instruments are used).
- If the Ct value is present for C– and/or for NCA in the FAM/Green, JOE/Yellow channels in the results grid. Test analysis must be repeated for all samples with Ct value defined in the FAM/Green and/or JOE/Yellow channel.
- If the Ct value for DNA calibrators (UG1 and UG2) is not detected in the FAM/Green

and JOE/Yellow channels, or if the difference between Ct values does not fall in the range specified in the *Important Product Information Bulletin*. PCR should be repeated for all samples.

- If human DNA is absent in the clinical material. Material sampling and PCR analysis should be repeated.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear). This may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

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

11. TRANSPORTATION

AmpliSens® *M.genitalium*-screen-titre-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® *M.genitalium*-screen-titre-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *M.genitalium*-screen-titre-FRT** PCR kit 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 °C when not in use.



PCR-mix-1-FL *M.genitalium*-screen-titre is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens[®] *M.genitalium*-screen-titre-FRT** PCR kit is the following:

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml ³
Urogenital swabs ⁴	DNA-sorb-AM	1x10 ³
Urine (first portion) ⁵	DNA-sorb-AM	2x10 ³

The linear measurement range for quantitative detection of this microorganism is 10³-10⁷ GE/ml.

13.2. Specificity

The analytical specificity of **AmpliSens[®] *M.genitalium*-screen-titre-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. Nonspecific reactions were absent while testing human DNA samples and DNA panel of the following microorganisms: *Gardnerella vaginalis*, *Lactobacillus* spp., *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp., *Candida albicans*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Neisseria* spp., *Ureaplasma* spp., *Mycoplasma hominis*, *Trichomonas vaginalis*, *Treponema pallidum*, *Toxoplasma gondii*, HSV of 1 and 2 types, CMV and HPV. The clinical specificity of **AmpliSens[®] *M.genitalium*-screen-titre-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 and Quantitation of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum*, *Ureaplasma urealyticum*" developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-

³ Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

⁴ Urogenital swabs are to be placed into the Transport medium for swabs (REF 956-CE, 987-CE) or Transport medium with mucolytic agent (REF 952-CE, 953-CE), Transport medium TM-EDEM (REF 1533-CE).














⁵ Treatment is required.

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[®] M.genitalium-screen-titre-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
RG	For work with Rotor-Gene 3000/6000 (Corbett Research)	IC	Internal control
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	iQ	For work with iCycler iQ and iQ5 (Bio-Rad)

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"