



For *in Vitro* Diagnostic Use

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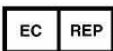
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AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE

AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT PCR kit is an *in vitro* nucleic acid amplification test for multiplex detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Trichomonas vaginalis* DNA in the clinical materials (urogenital swabs), rectum swabs, pharynx mucous membrane, urine sediment, conjunctiva samples, secrete of the prostate gland) by using real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION

N. gonorrhoeae/ C. trachomatis /Ureaplasma /M. genitalium detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific regions using special *N. gonorrhoeae/ C. trachomatis /Ureaplasma /M. 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[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT PCR kit is a qualitative test, which contains the Internal Control-FL (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[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-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). The 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[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT PCR kit is produced in 2 forms:

AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT PCR kit variant FRT, [REF](#) R-B61(RG).

AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT PCR kit variant FRT-100 F, [REF](#) R-B61-F(RG).

AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT PCR kit, variant FRT includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FL <i>N.gonorrhoeae/ C.trachomatis/ M.genitalium/ T.vaginalis</i>	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 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 protocol).

AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT PCR kit, variant FRT is intended for 110 reactions, including controls.

AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT PCR kit, variant FRT-100 F includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FL <i>N.gonorrhoeae /C.trachomatis/M.genitalium/T.vaginalis</i>	colorless, clear liquid	1.1	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 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 protocol).

AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT PCR kit, variant FRT-100 F is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA isolation 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™ 6000 (Corbett Research, Australia); Rotor-Gene™ Q (Qiagen, Germany) or equivalent).
- Disposable polypropylene microtubes for PCR with 0.2 ml (0.1) capacity (for example, Axygen, USA; Corbett Research, Australia; Qiagen, Germany).
- Refrigerator for temperature 2-8 °C.
- Deep-freezer with temperature no more than minus 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 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, 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 manufacturer's handbook [1]. It is recommended to read this handbook before starting work.

AmpliSens® *N. gonorrhoeae*/ *C. trachomatis*/ *M. genitalium*/ *T. vaginalis*-MULTIPRIME-FRT PCR kit is intended for analysis of DNA extracted by using DNA isolation kits from cervical or urethral scrapes (swabs), rectum swabs, pharynx mucous membrane, urine sediment (use the first part of the stream), conjunctiva samples 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.
- Other nucleic acid extraction kits, recommended by Federal State Institution of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being [2].



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

7.2. Preparing the PCR

Variant FRT

Total reaction volume - **30 µl**, volume of DNA sample - **10 µl**.

7.2.1. Preparing tubes for PCR

1. Prepare the required number of the tubes with **PCR-mix-1-FL *N.gonorrhoeae*/ *C.trachomatis*/ *M.genitalium*/ *T.vaginalis*** 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 of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *N.gonorrhoeae*/ *C.trachomatis*/ *M.genitalium*/ *T.vaginalis***.

Variant FRT-100 F

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

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 out N reactions (including 2 controls) mix in a new tube: **10·(N+1) µl of PCR-mix-1-FL *N.gonorrhoeae*/ *C.trachomatis*/ *M.genitalium*/ *T.vaginalis***, **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 effective for both variants.

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

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

Program the thermocycler according to **Manufacturer's manual, Guidelines** and Table 1.

Table 1

AmpliSens-1 RG program

Step	Temperature, °C	Time	Cycle repeats
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	

Fluorescence detection is on the 2-nd pass (**60 °C**) in Green, Yellow, Orange, Crimson and Red fluorometer channels.

8. DATA ANALYSIS

Accumulation of *Neisseria gonorrhoeae* DNA amplification product is detected in **Green** fluorescence channel, *Chlamydia trachomatis* DNA is detected in **Yellow** channel, *Mycoplasma genitalium* DNA is detected in **Orange** channel, *Trichomonas vaginalis* DNA is detected in **Crimson** channel, and **Internal Control DNA** is detected in the **Red** channel.

Results interpretation.

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

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

Results for controls

Table 2

Control	Stage for control	Ct channels Green, Yellow, Orange, Crimson	Ct channel Red	Interpretation
C-	DNA isolation	Neg	Pos (< boundary value) *	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< boundary value) *	Pos (< boundary value) *	OK

- The sample is considered to be positive for *Neisseria gonorrhoeae* if its Ct value is defined in the results grid (the fluorescence curve crosses the threshold line) in Green channel.
- The sample is considered to be positive for *Chlamydia trachomatis* if its Ct value is defined in the results grid (the fluorescence curve crosses the threshold line) in Yellow channel.
- The sample is considered to be positive for *Mycoplasma genitalium* if its Ct value is defined in the results grid (the fluorescence curve crosses the threshold line) in Orange channel.
- The sample is considered to be positive for *Trichomonas vaginalis* if its Ct value is defined in the results grid (the fluorescence curve crosses the threshold line) in Crimson channel.
- The sample is considered to be negative for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Trichomonas vaginalis* if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in Green, Yellow, Orange and Crimson channels and the Ct value doesn't exceed boundary value in the results grid in the Red channel.

* For Ct boundary values of the samples, Negative Control of Extraction and Positive Control of Amplification see **Appendix 1**.

9. TROUBLESHOOTING

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

- If no signal is detected for the sample or its Ct value exceeds boundary value in Green, Yellow, Orange, Crimson and Red channels and PCR reaction should be repeated. If the same result is obtained, the sample analysis should be repeated starting from the extraction stage.
- If no signal is detected for Positive Control of Amplification (C+) or its Ct value exceeds boundary value in Green, Yellow, Orange and Crimson channels, PCR reaction should be repeated for the samples without detected signal in the channels.
- If the positive signal in negative controls (C- or NCA) in the channels for detection of pathogen DNA is registered, test analysis must be repeated for the samples, which Ct value is defined.
- If no signal was detected in the channels for detection of pathogen DNA and for detection of Internal Control, the result is considered to be invalid. The sample should be examined repeatedly (PCR and detection).

10. STABILITY AND STORAGE

All components of the **AmpliSens® N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis-MULTIPRIME-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at the temperature 2-8 °C, when not in use. All components of the **AmpliSens® N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis-MULTIPRIME-FRT** PCR kit are to be stable until labeled expiration date.



PCR-mix-1-FL *N.gonorrhoeae/ C.trachomatis/ M.genitalium/ T.vaginalis* is to be kept away from light.



Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature no more than minus 16 °C.

11. SPECIFICATIONS

11.1. Sensitivity

Analytical Sensitivity of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Trichomonas vaginalis* DNA is not less than 5×10^2 genome equivalents per 1 ml of sample (GE/ml).



Analytical Sensitivity of each microorganism does not change even in the case of the high concentration of other microorganisms.

11.2. Specificity

Specificity of **AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT** PCR kit is ensured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. Specificity of **AmpliSens[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT** PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES

1. 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.
2. Guidelines "Real-time PCR detection of STIs and other reproductive tract infections", issued 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.

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[®] *N. gonorrhoeae/ C. trachomatis/ M. genitalium/ T. vaginalis*-MULTIPRIME-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS



Manufacturer



Temperature limitation



Use by



Batch code



For *in Vitro* Diagnostic Use



Version



Catalogue number



Internal Control



Contains sufficient for <n> tests



Caution, consult accompanying documents



Consult instructions for use



Negative Control of Amplification



Positive Control of Amplification



Negative Control of Extraction



Rotor-Gene