ECOLI

eSens

CE

IVD

For Professional Use Only

eSens Mycoplasma genitalium QL PCR kit

REF ES3003A

Instructions for Use

1 INTENDED USE

eSens Mycoplasma genitalium QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycoplasma genitalium* DNA in the clinical material (urogenital swabs, urine, and prostate gland secretion) using real-time hybridization-fluorescence detection of amplified products.

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

2 PRINCIPLE OF PCR DETECTION

Mycoplasma genitalium DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Mycoplasma genitalium* primers. In the real-time PCR, the amplified product is detected with the use of 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.

eSens Mycoplasma genitalium QL 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.

eSens Mycoplasma genitalium QL PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR:

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	**FAM**	**JOE**
DNA-target	*M. genitalium* DNA	Internal Control-FL (IC) DNA
Target gene	gyrB gene	Artificially synthesized sequence

3 CONTENT

eSens Mycoplasma genitalium QL PCR kit (ES30003A) contains:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-1-FL *Mycoplasma genitalium*	clear liquid from colorless to light lilac colour	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 (IC)** during the DNA extraction directly to the sample/lysis mixture.

eSens Mycoplasma genitalium QL PCR kit is intended for 110 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene tubes:

    - thin-walled 0.2-ml PCR tubes with domed caps if a plate-type instrument is used;
    - thin-walled 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 filters 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 inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- 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

eSens Mycoplasma genitalium QL PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (urogenital swabs; urine samples (sediment of the first portion of the morning specimen); prostate gland secretion).

7 WORKING CONDITIONS

eSens Mycoplasma genitalium QL PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction

- **DNA-sorb-AM** (K1-12-100-CE)

- For the automatic extraction

- **ePure STD DNA Extraction Kit (E2007)**

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL (IC).**

Please carry out nucleic acid extraction according to the manufacture´s instruction.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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

1. Thaw the tube with **PCR-mix-2-FRT** tube. Vortex the tubes with **PCR-mix-1-FL *Mycoplasma genitalium*, PCR-mix-2-FRT,** and **polymerase (TaqF),** and sediment the drops by short centrifugation (1-2 s).
   Take the required number of the tubes/stripes for amplification of DNA from clinical and control samples.
2. For N reactions (including 2 controls of amplification), add to a new tube:
   10*(N+1) µl of **PCR-mix-1-FL *Mycoplasma genitalium*;**
   5.0*(N+1) µl of **PCR-mix-2-FRT;**
   0.5*(N+1) µl of **polymerase (TaqF).**
   Mix the prepared mixture and sediment the drops by short centrifugation (1-2 s).

Transfer **15 µl** of the prepared mixture to each tube.

Steps 3 and 4 are required out in both variants.

3. Add **10 µl** of **DNA** obtained at the DNA extraction stage into the 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 (C+)** (to the tube labeled C+ (Positive Control of Amplification).
C–	Add **10 µl** of sample extracted from the **Negative Control (C–) reagent** to the tube labeled C– (Negative Control of Extraction).

8.2.2 Amplification

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

Table 2

eSens-1 amplification program

Step	**Rotor-type instruments** (e.g Rotor-Gene Q or equivalent)			**Plate-type instruments** (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.)		
	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 Fluorescence acquiring		60	30 s Fluorescence acquiring	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores. Other channels are enabled if several tests are simultaneously carried out in a single run.

2. Adjust the fluorescence channel sensitivity.
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.

8.3 Instrument Settings

Test settings for rotor-type instruments

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 5 FI to 10 FI	0.1	On	Off	0%
JOE/Yellow	from 4 FI to 8 FI	0.1	On	Off	5%

Test settings for plate-type instruments

Set the heating/cooling ***Ramp Rate* 2,5 °C/s**.

Channel	Threshold
FAM, JOE/HEX	For each channel in ***Log Scale*** set the threshold line al the level of 10-20 % of maximum fluorescence obtained for the Positive Control of Amplification (C+) in the last amplification cycle

9 DATA ANALYSIS

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

- The signal of the *Mycoplasma genitalium* DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the IC amplification product is detected in the channel for the JOE 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:

- *Mycoplasma genitalium* DNA is **detected** in a sample if the Ct value is determined in the result grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of exponential growth of fluorescence.
- *Mycoplasma genitalium* DNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the result grid (the fluorescence curve does not cross the threshold line) in the channel for the FAM fluorophore, whereas the *Ct* value determined in the channel for the JOE fluorophore does not exceed the specified boundary value.
- The result is **invalid** if the *Ct* value is not determined (absent) in the channel for the FAM fluorophore, whereas the *Ct* value in the channel for the JOE fluorophore is not determined (absent) or exceeds the specified boundary value. In such cases PCR should be repeated for this sample.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 3 and 4).

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	**JOE**
C–	DNA extraction	Absent	<boundary value
NCA	PCR	Absent	Absent
C+	PCR	<boundary value	<boundary value

Table 4

Boundary *C*t values

Sample	Rotor-type instrument		Plate-type instrument	
	Channel for fluorophore			
	FAM	**JOE**	**FAM**	**JOE**
C+	33	30	36	33
C-	Ct is absent	30	Ct is absent	33
NCA	Ct is absent		Ct is absent	
Test samples, C-	-	30	-	33

10 TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of amplification (C+) in the channel for the FAM fluorophore is greater than the specified boundary value or absent. The amplification should be repeated for all the samples in which the *Mycoplasma genitalium* DNA was not detected.
2. The *Ct* value is determined for the Negative Control of Extraction (C–) and/or the Negative Control of Amplification (NCA) in the channel for the FAM fluorophore. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which *Mycoplasma genitalium* DNA was detected.

11 TRANSPORTATION

eSens Mycoplasma genitalium QL PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens Mycoplasma genitalium QL 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 **eSens Mycoplasma genitalium QL PCR kit** are stable until the expiry date stated on the label. PCR kit can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, PCR kit should be unpacked in accordance with the

storage temperatures for each component. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FL Mycoplasma genitalium is to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

The analytical sensitivity of **eSens Mycoplasma genitalium QL PCR kit** is following

Clinical material	**Transport medium**	**DNA extraction kit**	**Analytical sensitivity, GE/ml***
Urogenital swabs	Transport Medium for Swabs or Transport Medium with Mucolytic Agent	DNA-sorb-AM ePure STD DNA Extraction kit	$1x10^3$
Urine (pretreatment is required)	—	DNA-sorb-AM ePure STD DNA Extraction kit	$2x10^3$

*The quantity of genome equivalents of microorganism per 1 ml of the sample from transport medium.

13.2 Specificity

The analytical specificity of **eSens Mycoplasma genitalium QL PCR kit** is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

Nonspecific reactions were absent while testing human DNA and DNA panels of the following microorganisms: *Mycoplasma hominis; Ureaplasma urealyticum*, *Ureaplasma parvum; Gardnerella vaginalis; Lactobacillus* spp.; *Escherichia coli; Staphylococcus aureus; Streptococcus pyogenes*, *Streptococcus agalactiae; Candida albicans; Neisseria flava, Neisseria subflava, Neisseria sicca, Neisseria mucosa, Neisseria gonorrhoeae; Chlamydia trachomatis; Trichomonas vaginalis; Treponema pallidum; Toxoplasma gondii; HSV* types 1 and 2; *CMV;* and *HPV.*

The clinical specificity of **eSens Mycoplasma genitalium QL PCR kit** was confirmed in laboratory clinical trials.

14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

15 KEY TO SYMBOLS USED

REF	Catalogue number
LOT	Batch code
IVD	*In vitro* diagnostic medical device
VER	Version
	Temperature limit
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community

	Caution
	Contains sufficient for <n> tests
	Use-by Date
	Consult instructions for use
	Keep away from sunlight
NCA	Negative control of amplification
C-	Negative control of extraction
C+	Positive control of amplification
IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

Ecoli Dx, s.r.o. , Purkyňova 74/2

110 00 Praha 1, Česká republika
Tel: +420 325 209 912

Mobil: +420 739 802 523