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For Professional Use Only

# eSens Ureaplasma sp. QL PCR kit

**REF ES3007A**

Instructions for Use



## 1 INTENDED USE

**eSens Ureaplasma sp. QL PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection of *Ureaplasma* species *(U. parvum* and *U. urealyticum)* DNA in the clinical material (urogenital swabs, urine samples, 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

*Ureaplasma* species *(U. parvum* and *U. urealyticum)* detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using special 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.

**eSens Ureaplasma sp. 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 Ureaplasma sp. QL PCR kit** uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. In eSens Ureaplasma sp. QL PCR kit, “hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using a wax layer. Wax melts and reaction components mix only at 95 °C. In eSens Ureaplasma sp. QL PCR kit, “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 eSens Ureaplasma sp. QL 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 | *Ureaplasma* spp. DNA | Internal Control (IC) DNA |
| Target gene | Urease gene | Artificially synthesized sequence |

# 3 CONTENT

**eSens Ureaplasma sp. QL PCR kit (ES3007A)** includes:

| **Reagent** | **Description** | **Volume, ml** | **Quantity** |
|---|---|---|---|
| **PCR-mix-1-FL *Ureaplasma* spp.** | 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 Ureaplasma sp. 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 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 Ureaplasma sp. QL PCR kit** is intended for analysis of DNA extracted with the use of 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 Ureaplasma sp. 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).**

In the extraction procedure it is necessary to carry out the control reactions as follows:

**C–** **Add 100 µl of Negative Control (C–)** to the tube labeled C– (Negative control of Extraction).

**NOTE:** Extract DNA according to the instructions provided by the manufacturer.

## 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**. Vortex the tubes with **PCR-mix-1-FL *Ureaplasma* spp., PCR-mix-2-FRT,** and **polymerase (TaqF)** and sediment the drops by short centrifugation (1-2 s). Take the required quantity of the tubes/stripes for amplification of DNA obtained from clinical and control samples.
2. For N reactions (including 2 controls of amplification) mix in a new tube:
   **10*(N+1) µl** of **PCR-mix-1-FL *Ureaplasma* spp.;**
   **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 into each tube.
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 a sample extracted from the Negative Control (C–) 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**

| | **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.) | | |
|---|---|---|---|---|---|---|
| Step | Temperature, °C | Time | Cycles | Temperature, °C | Time | Cycles |
| 1 | 95 | 15 min | 1 | 95 | 15 min | 1 |
| 2 | 95 | 5 s | 5 | 95 | 5 s | 5 |
| | 60 | 20 s | | 60 | 20 s | |
| | 72 | 15 s | | 72 | 15 s | |
| 3 | 95 | 5 s | 40 | 95 | 5 s | 40 |
| | 60 | 20 s<br>fluorescent signal detection | | 60 | 30 s<br>fluorescent<br>signal detection | |
| | 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.

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<br>JOE/HEX | For each channel in ***Log Scale*** set the threshold line at 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 *Ureaplasma* spp. DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the Internal Control 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:

- *Ureaplasma* spp. 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 fluorescence growth of fluorescence.
- *Ureaplasma* spp. 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 results grid 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 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 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 *Ct* 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 | - | 30 | - | 33 |

## 10 TROUBLESHOOTING

Results of 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 *Ureaplasma* spp. 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 *Ureaplasma* spp. DNA was detected.

## 11 TRANSPORTATION

**eSens Ureaplasma sp. 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 Ureaplasma sp. 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 Ureaplasma sp. QL PCR kit** are stable until the expiry date stated on the label. **eSens Ureaplasma sp. QL PCR kit** can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, **eSens Ureaplasma sp. QL 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 temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FL Ureaplasma spp. is be kept away from light.

# 13 SPECIFICATIONS

## 13.1 Sensitivity

The analytical sensitivity of **eSens Ureaplasma sp. QL PCR kit** is specified in the table below.

| **Clinical material** | **Transport medium** | **DNA extraction kit** | **Analytical sensitivity, GE/ml*** |
|---|---|---|---|
| Urogenital swabs | Transport Medium with Mucolytic Agent | DNA-sorb-AM<br>ePure STD DNA Extraction Kit | 1 x $10^3$ |
| Urine** | — | DNA-sorb-AM<br>ePure STD DNA Extraction Kit | 2 x $10^3$ |

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

**Pretreatment is required.

## 13.2 Specificity

The analytical specificity of **eSens Ureaplasma sp. QL PCR kit** is ensured by the 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.

Nonspecific reactions were absent while testing human DNA samples and DNA panel of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Mycoplasma hominis, Mycoplasma genitalium, 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 Ureaplasma sp. 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 | | |

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