ECOLI

eSens

CE

IVD

For Professional Use Only

eSens Pneumocystis jirovecii QL PCR kit

REF ES3803B

Instructions for Use

1 INTENDED USE

eSens Pneumocystis jirovecii QL PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Pneumocystis jirovecii (carinii)* DNA in the clinical material (bronchoalveolar lavage, sputum, oropharyngeal and tracheal aspirates, lung biopsy material, oropharyngeal washes and swabs) using real-time hybridization-fluorescence detection.

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

2 PRINCIPLE OF PCR DETECTION

Pneumocystis jirovecii (carinii) DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Pneumocystis jirovecii (carinii)* 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 Pneumocystis jirovecii QL PCR kit is a qualitative test based on the use of an endogenous control, the β-globin gene. The DNA target selected as an endogenous internal control is a human genome fragment that is present in sample in a sufficient quantity equivalent to that of cells in the sample. The use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and amplification) but also to assess the adequacy of sampling and storage of clinical material.

eSens Pneumocystis jirovecii QL PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” 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 dUTP. 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 dUTP 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	DNA fragment of β-globin gene (IC Glob)	*Pneumocystis jirovecii (carinii)* DNA
Target gene	fragment of human β-globin gene	gene fragment of *Pneumocystis jirovecii (carinii)* mitochondrial rRNA large subunit

3 CONTENT

eSens Pneumocystis jirovecii QL PCR kit (ES3803B) includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-1-FRT *P.jirovecii* / Glob	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Positive Control DNA *P.jirovecii* and human DNA (C+*P.jirovecii* and human DNA)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	0.5	1 tube

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

eSens Pneumocystis jirovecii QL PCR kit is intended for 60 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- Reagent for pretreatment of viscous fluids (sputum, bronchoalveolar lavage).
- Saline solution or PBS (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM sodium monophosphate, and 2 mM potassium diphosphate (pH 7.5 ± 0.2)) for pretreatment of sputum and biopsy material.
- 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:
- tightly closed or screwed 1.5-ml tubes for pretreatment
- tightly closed or screwed 2.0-ml tubes for pretreatment
- screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
    - 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 barriers 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 samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- 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 Pneumocystis jirovecii QL PCR kit is intended for analysis of *Pneumocystis jirovecii (carinii)* DNA extracted with DNA extraction kits from the clinical material (bronchoalveolar lavage, sputum, oropharyngeal and tracheal aspirates, lung biopsy material, oropharyngeal washes and swabs)

1. *Bronchoalveolar lavage, oropharyngeal and tracheal aspirates* samples should be placed into a sterile disposable tube or container. The material must be pretreated for the analysis. Thoroughly resuspend the sample and transfer 1 ml of the material into an Eppendorf tube using a filter tip. Centrifuge the tube for 10 min at 7,000 g (8,000-10,000 rpm in a 24-well centrifuge or 10,000–13,000 rpm in a 12-well centrifuge). Carefully remove and discard the supernatant using a filter tip and leaving 200 µl of the liquid on the sediment. Resuspend the sample on vortex.

   Bronchoalveolar lavage, oropharyngeal and tracheal aspirates and pretreated material can be stored:

   - at 2–8 °C for 1 day;
   - at the temperature not more than minus 16 °C for 7 days;
   - at the temperature not more than minus 68 °C for a long time.

   NOTE: Only one freeze–thaw cycle of clinical material is allowed.

2. *Sputum*. Sputum is collected into a sterile disposable container after preliminarily rinsing the mouth with water. The material must be pretreated for example by mucolysin for the analysis. Samples can be stored:

   - at 2–8 °C for 3 days;
   - at the temperature not more than minus 16 °C for 7 days;
   - at the temperature not more than minus 68 °C for a long time.

   NOTE: Only one freeze–thaw cycle of clinical material is allowed.

3. *Biopsy and autopsy material samples.* Tissue samples are excised from the areas of presumable pathogen location, from damaged tissue, or from areas adjacent to the damaged areas. Tissue fragments not more than 5 mm in diameter are placed in a sterile disposable 2-ml Eppendorf tube containing 0.5 ml of **Transport Medium with Mucolytic Agent** (952-CE)

   Samples can be stored:

   - at room temperature for 6 h;
   - at 2–8 °C for 3 days;
   - at the temperature not more than minus 16 °C for a long time.

   For the analysis of larger fragments of tissues, place a sample in a sterile porcelain mortar. Add an equal volume of saline or PBS. Thoroughly homogenize the sample with a pestle. Transfer 100 µl of the prepared suspension to a sterile tube for DNA extraction. Samples can be stored at the temperature not more than minus 16 °C.

4. *Oropharyngeal washes and swabs* placed in a tube with transport medium

   Samples can be stored:

   - at 2–8 °C for 1 day;
   - at the temperature not more than minus 68 °C for 1 year.

7 WORKING CONDITIONS

eSens Pneumocystis jirovecii 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

- **RIBO-prep** (K2-9-Et-50-CE).

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

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

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 type of tubes depends on the type of PCR real-time instrument.

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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

1. Preparation of **PCR-mix-2-FRT** and **polymerase (TaqF)** mixture:

   Transfer the entire content of the tube **(30 µl)** with **polymerase (TaqF)** into the tube containing **300 µl** of **PCR-mix-2-FRT** and vortex carefully avoiding foaming. Label the tube by the date of preparation.

   NOTE: The prepared mixture is intended for analysis of 60 samples.

   Store the prepared mixture at 2–8 °C for 3 months and use it as needed.

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

2. Prepare the reaction mixture. Note that, for testing even one experimental DNA sample, two controls of amplification (one positive and one negative) should be carried out. It is recommended to mix reagents for an even number of reactions to ensure more accurate dispensing.
3. Mix in a new tube **PCR-mix-1-FRT *P.jirovecii* / Glob** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF**), which was prepared earlier. Prepare the required amount of the mixture proceeding from the volumes required for one reaction:

- 10 µl of PCR-mix-1-FRT *P.jirovecii* / Glob;
- 5 µl of the mixture of PCR-mix-2-FRT and polymerase (TaqF).

4. Calculate the volume of the reaction mixture for the required number of reactions including the testing of the clinical and control samples according to the scheme of the reaction mixture preparation (see Table 2).

Table 2

Scheme of reaction mixture preparation

Number of clinical samples including controls	PCR-mix-1-FRT *P. jirovecii* / Glob, µl	PCR-mix-2-FRT + polymerase (TaqF), µl
1	40	20
2	50	25
3	60	30
4	70	35
5	80	40
6	90	45
7	100	50
8	110	55
9	120	60
10	130	65
11	140	70
12	150	75
13	160	80
14	170	85
15	180	90
16	190	95
17	200	100
18	210	105
19	220	110
20	230	115
21	240	120
22	250	125
23	260	130

24	270	135
25	280	140
26	290	145
27	300	150
28	310	155
29	320	160
30	330	165
31	340	170
32	350	175
33	360	180
34	370	185

5. Take the required number of tubes for amplification of DNA extracted from clinical and control samples. The type of tubes depends on the PCR instrument used. Add 15 µl of the prepared reaction mixture into each tube.
6. Add **10 µl** of **DNA** samples obtained at the DNA extraction stage into the prepared tubes with the reaction mixture.
7. Carry out the control amplification reactions:

NCA	Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).
C+	Add **10 µl** of **Positive Control DNA *P.jirovecii* and human DNA (C+*P.jirovecii* and human DNA)** to the tube labeled C+ (Positive Control of Amplification).
C–	Add **10 µl** of **the 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 3

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 fluorescent signal detection		60	30 s fluorescent 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.
5. Analyze results after the amplification program is completed.

8.3 Instrument Settings

Test settings for Rotor-Gene Q

Channel	**Calibrate/Gain Optimisation**	**Threshold**	**Dynamic tube**	**Slope Correct**	**More Settings/ Outlier Removal**
FAM/Green	from 5FI to 10FI	0.03	on	on	10 %
JOE/Yellow	from 5FI to 10FI	0.03	on	on	10 %

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 **β-globin gene DNA (Internal Control Glob (IC Glob))** amplification product is detected in the channel for the FAM fluorophore.
- The signal of the ***Pneumocystis jirovecii (carinii)* DNA** 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:

- *Pneumocystis jirovecii (carinii)* DNA is **detected** in a sample if the *Ct* value determined in the results grid in the channel for the JOE fluorophore is less than the specified boundary *Ct* value. Moreover, the fluorescence curve should cross the threshold line in the area of the exponential growth of fluorescence.
- *Pneumocystis jirovecii (carinii)* DNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the results grid (the fluorescence curve does not cross the threshold line) in the channel for the JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the specified boundary *Ct* value.
- The result is **invalid** if the *Ct* value of a sample in the channel for the JOE fluorophore is not determined (absent) and the *Ct* value in the channel for the FAM fluorophore is not determined (absent) or greater than the boundary *Ct* value. In such cases, the PCR analysis should be repeated.
- The result is **equivocal** if the *Ct* value in the channel for the JOE fluorophore exceeds the boundary *Ct* value. The PCR analysis of this sample should be repeated in duplicate. If a reproducible positive *Ct* value is obtained, the sample is considered positive. If the result is not reproduced in duplicate, the sample is considered equivocal.

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 4 and 5).

Table 4

Results for controls

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

Table 5

Boundary *Ct* values

	Rotor-type instrument		**Plate-type instrument**	
Sample	**Channel for fluorophore**			
	FAM	**JOE**	**FAM**	**JOE**
C-	Ct value is absent		*Ct* value is absent	
NCA	Ct value is absent		Ct value is absent	
C+	23	23	26	26
Clinical samples	30	35	30	35

10 TROUBLESHOOTING

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

1. If the *Ct* value is determined for the Negative Control of extraction (C–) and for the Negative Control of Amplification (NCA) in the channels for the FAM and JOE fluorophores, it indicates the contamination of reagent or samples. The PCR analysis (beginning with the DNA extraction stage) should be repeat for all samples in which *Pneumocystis jirovecii (carinii)* DNA was detected.
2. If the *Ct* value is absent or exceeds the boundary *Ct* value for the Positive Control of Amplification (C+), the PCR analysis of all samples should be repeated starting from the PCR stage.
3. If the *Ct* values for the clinical samples in the channel for the FAM fluorophore are absent in the results grid, this suggest the extraction stage failure. The PCR analysis of these samples should be repeated starting from extraction stage.
4. If *Ct* values for the analyzed sample exceed the boundary *Ct* values in the channels for the FAM and JOE fluorophores, the analysis of this sample should be repeated starting from the DNA extraction stage. High *Ct* values can be due to the loss of DNA or the presence of inhibitors

11 TRANSPORTATION

eSens Pneumocystis jirovecii 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 Pneumocystis jirovecii QL PCR kit** are to be stored at 2–8 °C when not in use (except for polymerase (TaqF), PCR-mix-2-FRT and PCR-mix-1-FRT *P.jirovecii* / Glob). All components of the **eSens Pneumocystis jirovecii QL PCR kit** are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Polymerase (TaqF), PCR-mix-2-FRT and PCR-mix-1-FRT P.jirovecii / Glob are to be stored at the temperature from minus 24 to minus 16 °C when not in use.

NOTE: PCR-mix-1-FRT P.jirovecii / Glob is to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

The analytical sensitivity of the **eSens Pneumocystis jirovecii QL PCR kit** is given the table below.

Clinical material	DNA extraction kit	Analytical sensitivity, copies/ml
Bronchoalveolar lavage; sputum; oropharyngeal and tracheal aspirates; oropharyngeal washes and swabs; lung biopsy material	RIBO-prep	500

13.2 Specificity

The analytical specificity of **eSens Pneumocystis jirovecii 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 in tests of human DNA samples and DNA samples of the following organisms: fungi (*Penicillium auzontiag, P.brevicompactum*; *Ulocladium boffcytus*; *Mucor racemosus*, *M.plumbeus*; *Aspergillus versicolor*, *A.niger*, *A.flavus*, *A.fumigatus*; *Cryptococcus neoformans*), viruses (*Epstein-Barr virus; Herpes simplex virus* types I and II; *Herpes virus* types 6 and 8, *Varicella-Zoster virus, Parvovirus B19,* and others), bacteria (*Streptococcus pyogenes*, *S.agalactiae; Staphylococcus aureus,* and others).

The clinical specificity of the **eSens Pneumocystis jirovecii 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		
02_12/2025	9 DATA ANALYSIS	Table 5 was changed.

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