ECOLI

eSens

CE

IVD

For Professional Use Only

eSens N.meningitidis/H.influenzae/ S.pneumoniae QL PCR kit

REF ES3440B

Instructions for Use

1 INTENDED USE

eSens N.meningitidis/H.influenzae/S.pneumoniae QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Neisseria meningitidis*, *Haemophilus influenzae,* and *Streptococcus pneumoniae* DNA in clinical material (cerebrospinal fluid) 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

Neisseria meningitidis, *Haemophilus influenzae,* and *Streptococcus pneumoniae* detection by the polymerase chain reaction (PCR) is based on the multiplex amplification of the pathogen genome specific region in two tubes using specific primers. In real-time PCR, the amplified product is detected using 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 N.meningitidis/H.influenzae/S.pneumoniae *QL PCR kit* is a qualitative test that contains the Internal Control (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 N.meningitidis/H.influenzae/S.pneumoniae *QL PCR kit* uses "hot-start," which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using a 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**
Name of PCR-mix-1-FEP/FRT	**DNA-target**	
***Neisseria meningitidis* / STI**	Internal Control-FL (IC) DNA	*Neisseria meningitides* DNA
Streptococcus pneumoniae* / *Haemophilus influenzae	*Streptococcus pneumoniae* DNA	*Haemophilus influenza* DNA
Name of PCR-mix-1-FEP/FRT	**Target gene**	
***Neisseria meningitidis* / STI**	Artificially synthesized sequence	ctr A
Streptococcus pneumoniae* / *Haemophilus influenzae	ply	bex A

3 CONTENT

eSens N.meningitidis/H.influenzae/S.pneumoniae QL PCR kit (ES3440B) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae*	clear liquid from colorless to light lilac colour	0.6	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
Positive Control DNA *Neisseria meningitidis*-Flu (C+$_{N.meningitidis}$)	colorless clear liquid	0.1	1 tube
Positive Control DNA *Haemophilus influenzae* (C+$_{H.influenzae}$)	colorless clear liquid	0.1	1 tube
Positive Control DNA *Streptococcus pneumoniae* (C+$_{S.pneumoniae}$)	colorless clear liquid	0.1	1 tube
Positive Control STI-88 (CS+)	colorless clear liquid	0.1	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 procedure directly to the sample/lysis mixture

eSens N.meningitidis/H.influenzae/S.pneumoniae QL PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- 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 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 PCR tubes (0.1- or 0.2-ml):
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 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 N.meningitidis/H.influenzae/S.pneumoniae QL PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (cerebrospinal fluid).

Obtain cerebrospinal fluid (CSF) using disposable needles and collect it in an amount of not less than 1.0.ml into the disposable dry 2.0-ml plastic tubes. Tightly close the tubes, avoiding clearance and crumpling of the tube caps. Mark the tubes.

Samples can be stored at the room temperature for 6 hours, at 2-8 °C for 1 day, at the temperature below minus 16°C for 1 month, and at the temperature minus 68°C for a long time.

NOTE: Only one freeze-thaw cycle of the material is allowed.

7 WORKING CONDITIONS

eSens N.meningitidis/H.influenzae/S.pneumoniae 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-B** **REF** K1-2-50-CE;
- **RIBO-prep** **REF** K2-9-Et-50-CE;

- For the automatic extraction

- **ePure Bacterial DNA Extraction Kit (E2006)**

It is recommended to use RIBO-prep extraction kit for clinical samples that are simultaneously tested for *enterovirus* infections.

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

Extract DNA according to the manufacturer's protocol.

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**.

NOTE: Reaction mixture components should be mixed just before analysis with calculating for the required number of reactions (test and control samples) according to the Table 2. Note that even for analysis of one test DNA sample, it is necessary to carry out all controls of the PCR stage: Positive Control of Amplification (C+) and Negative Control of Amplification (NCA) for each PCR-mix. It is recommended to mix the reagents for an even reaction number to ensure more exact dosage

1. Thaw all the reagents, vortex the tubes thoroughly, and sediment drops from walls of tubes.
2. Take the required number of tubes for amplification of the DNA from clinical and control samples.
3. To prepare the reaction mixture, mix one of the PCR-mixes-1 (**PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI** or **PCR-mix-1-FEP/FRT *Streptococcus pneumoniae / Haemophilus influenzae)***, **PCR-mix-2-FRT,** and **polymerase (TaqF)** according to Table 2. Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 2

Scheme of reaction mixture preparation

	Reagent volume for the specified number of reactions (µl)		
Reagent volume per one reaction (µl)	**10.00**	**5.00**	**0.50**
Number of reactions*	**PCR-mix-1-FEP/FRT**	**PCR-mix-2-FRT**	**Polymerase (TaqF)**
6	**60**	**30**	**3.0**
8	**80**	**40**	**4.0**
10	**100**	**50**	**5.0**
12	**120**	**60**	**6.0**
14	**140**	**70**	**7.0**
16	**160**	**80**	**8.0**
18	**180**	**90**	**9.0**
20	**200**	**100**	**10.0**
22	**220**	**110**	**11.0**
24	**240**	**120**	**12.0**
26	**260**	**130**	**13.0**
28	**280**	**140**	**14.0**
30	**300**	**150**	**15.0**
32	**320**	**160**	**16.0**

***** Number of test samples including controls of extraction (N), controls of amplification and one extra reaction (N+3+1).

4. Transfer **15 µl** of the prepared mixture to each tube. Dispose of the unused reaction mixture.
5. Using tips with aerosol filter, add **10 µl** of **DNA** obtained at the DNA extraction stage.

NOTE: Avoid transferring sorbent together with the DNA sample extracted by DNA-sorb-B kit.

6. Carry out the control amplification reactions:

NCA	–	Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
C+*N.meningitidis*	–	Add **10 µl** of **Positive Control DNA *Neisseria meningitidis*-Flu** (for **PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI**) to the tube labeled C+*N.meningitidis* (Positive Control of Amplification).
CS+	–	Add **10 µl** of **Positive Control STI-88** (for **PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI**) to the tube labeled CS+ (Positive Control of Amplification).
C+*S.pneumoniae*	–	Add **10 µl** of **Positive Control DNA *Streptococcus pneumoniae*** (for **PCR-mix-1-FEP/FRT *Streptococcus pneumoniae / Haemophilus influenzae***) to the tube labeled C+*S.pneumoniae* (Positive Control of Amplification).
C+*H.influenzae*	–	Add **10 µl** of **Positive Control DNA *Haemophilus influenzae*** (for **PCR-mix-1-FEP/FRT *Streptococcus pneumoniae / Haemophilus influenzae***) to the tube labeled C+*H.influenzae* (Positive Control of Amplification).
C–	–	Add **10 µl** of **the sample extracted from the Negative Control 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

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**
Hold	**95**	15 min	1	**95**	15 min	1
Cycling	**95**	10 s	45	**95**	10 s	45
	56	20 s Fluorescence acquiring		**56**	25 s Fluorescence acquiring	
	72	10 s		**72**	10 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.
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

Rotor-Gene Q

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 5FI to 10FI	0.05	On	On	10 %
JOE/Yellow	from 5FI to 10FI	0.05	On	On	5-10 %

Test settings for plate-type instruments

CFX96

Note: Set ***Ramp Rate* 2,5 °C/s** by clicking the ***Step Options*** button for each step of cycling.

Channel	Threshold
FAM HEX	Set the threshold line in the logarithmic scale at the level of 10-20 % of maximum fluorescence obtained for the Positive control 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 (for the FAM and JOE fluorophores).

Table 4

Correspondence between detection channels and pathogens

Channel for the fluorophore	PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI	PCR-mix-1-FEP/FRT *Streptococcus pneumoniae / Haemophilus influenzae*
FAM	Internal Control-FL DNA	*Streptococcus pneumoniae* DNA
JOE	*Neisseria meningitidis* DNA	*Haemophilus influenzae* DNA

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 for the DNA sample in the corresponding column of the results grid.

Results should be interpreted in accordance with Table 5 and Table 6.

Table 5

Result interpretation

PCR-mix-1	***Ct* value in the channel for fluorophore**		**Result**
	FAM	**JOE**	
PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI	**< boundary value**	> boundary value	*Neisseria meningitidis* DNA is not detected
	> boundary value or < boundary value	**< boundary value**	*Neisseria meningitidis* DNA is detected
	> boundary value	> boundary value	**Invalid** result Repeat extraction and PCR
PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae*	**< boundary value**	> boundary value	*Streptococcus pneumoniae* DNA is detected
	> boundary value	**< boundary value**	*Haemophilus influenzae* DNA is detected
	> boundary value	> boundary value	*Streptococcus pneumoniae* and *Haemophilus influenzae** DNA are not detected

* If the *Ct* value detected in the channel for FAM fluorophore is less than the boundary value (with the use of **PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI**).

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 6).

Table 6

Results for controls

PCR-mix-1	Control	Stage for control	*Ct* value in the channel for fluorophore	
			FAM	JOE
PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI	C-	DNA extraction	**< boundary value**	> boundary value
	NCA	PCR	> boundary value	> boundary value
	C+$_{N.meningitidis}$	PCR	> boundary value	**< boundary value**
	CS+	PCR	**< boundary value**	> boundary value
PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus*	C-	DNA extraction	> boundary value	> boundary value
	NCA	PCR	> boundary value	> boundary value
	C+$_{S.pneumoniae}$	PCR	**< boundary value**	> boundary value
	C+$_{H.influenzae}$	PCR	> boundary value	**< boundary value**

Boundary *Ct* values

Sample	Rotor-type instrument		Plate-type instrument	
	Channel			
	FAM	JOE	FAM	JOE
CS+	38	-	40	-
C+$_{N.meningitidis}$	-	38	-	40
C+$_{S.pneumoniae}$	38	-	40	-
C+$_{H.influenzae}$	-	38	-	40
C-	38	-	40	-
Clinical samples	38	38	40	40

10 TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Controls of Amplification (C+) in the channels for the FAM or JOE fluorophores is greater than the boundary *Ct* value, the amplification and detection should be repeated for all samples in which *Ct* value determined in the channels for the FAM or JOE fluorophores was greater than the boundary *Ct* value for required PCR-mix-1.
2. If the *Ct* value determined for the Negative Control of Extraction (C-) (except for PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI in the channel for FAM fluorophore) and/or Negative Control of Amplification (NCA) (in all channels) is less than the boundary *Ct* value, PCR-analysis

should be repeated (beginning with DNA extraction) for all samples in which target pathogen DNA was detected.

11 TRANSPORTATION

eSens N.meningitidis/H.influenzae/S.pneumoniae 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 N.meningitidis/H.influenzae/S.pneumoniae QL PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI, PCR-mix-1-FEP/FRT *Streptococcus pneumoniae / Haemophilus influenzae,* PCR-mix-2-FRT, and polymerase (TaqF)). All components of the **eSens N.meningitidis/H.influenzae/S.pneumoniae QL PCR kit** are stable until the expiry date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI,
PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae*,
PCR-mix-2-FRT, and Polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C when not in use.

NOTE: PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI and
PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae*
are to be kept away from light

13 SPECIFICATIONS

13.1 Sensitivity

Clinical material	Nucleic acid extraction kit	Pathogen	Analytical sensitivity, GE/ml*
Cerebrospinal fluid	RIBO-prep	*Neisseria meningitidis*	$1x10^3$
	DNA-sorb-B	*Haemophilus influenzae*	
	ePure Bacterial DNA Extraction Kit	*Streptococcus pneumoniae*	

***** Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.

13.2 Specificity

The analytical specificity of **eSens N.meningitidis/H.influenzae/S.pneumoniae QL 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.

The specificity was proved on the following strains of microorganisms: *Enterobacter aerogenes; Enterobacter cloacae; Enterococcus faecalis* (GISK 29212); *Escherichia coli* (NCTC 9001); *Escherichia coli* (ATCC 25922); *Haemophilus parainfluenzae; Haemophilus Haemolyticus; Klebsiella oxytoca; Klebsiella pneumoniae; Listeria monocytogenes; Moraxella catarrhalis; Neisseria cinereae; Neisseria elongate; Neisseria flavescens; Neisseria gonorrhoeae; Neisseria mucosa; Neisseria sicca; Neisseria subflava; Pantoea agglomerans; Proteus mirabilis; Pseudomonas aeruginosa* (ATCC 27853); *Salmonella*

enteritidis (GISK 1137); *Salmonella typhi* (Central Public Health Laboratory (London) 5715); *Shigella flexneri* 2a (GISK 1270); *Shigella sonnei* (GISK 9090); *Staphylococcus aureus* (ATCC 25923); *Staphylococcus saprophyticus* (ATCC 15305); *Streptococcus pneumonia; Streptococcus agalactiae; Streptococcus milleri; Streptococcus mitis; Streptococcus mutans; Streptococcus pyogenes; Streptococcus salivarius; Streptococcus sanguis; Streptococcus suis; Streptococcus viridians; Yersinia enterocolitica; Yersinia pseudotuberculosis*. The analytical specificity was also confirmed by testing human DNA. Non-specific results were not detected.

The clinical specificity of **eSens N.meningitidis/H.influenzae/S.pneumoniae 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		Caution
LOT	Batch code		Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C-**	Negative control of extraction
		C+ ***N.meningitidis*** **C+** ***H.influenzae*** **C+** ***S.pneumoniae***	Positive controls 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