



For Professional Use Only

eSens *Streptococcus agalactiae* QT PCR kit

REF ES3809A

Instructions for Use

1 INTENDED USE

eSens *Streptococcus agalactiae* QT PCR kit is an *in vitro* nucleic acid amplification test for detection and quantitative analysis of *Streptococcus agalactiae* DNA in the clinical material (blood plasma, cerebrospinal fluid (CSF), oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs) using the polymerase chain reaction (PCR) with real-time fluorescence hybridization detection.

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

2 PRINCIPLE OF PCR DETECTION

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

The DNA extraction of cell-free biological material (blood plasma, CSF) is conducted with the aid of the **Internal Control STI-87 (IC)**. It must be used in the extraction procedure in order to control the extraction process of each individual sample. During the DNA extraction of cell containing clinical material, the DNA of the human genome (Endogenous Internal Control) is being amplified. The Endogenous Internal Control (**IC Glob**) allows not only to control the test stages (DNA extraction and PCR amplification) but also to assess the adequacy of sampling and storage of clinical material.

eSens Streptococcus agalactiae QT PCR kit uses “hot-start”, which greatly reduces the frequency of non-specifically primed reactions. “Hot-start” is guaranteed by 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	ROX
DNA-target	DNA fragment of β -globin gene (IC Glob)	<i>Streptococcus agalactiae</i> DNA	Internal Control STI-87L (IC) DNA
Target gene	β -globin gene	DNA section of the non-structural repeating sip gene (surface Sip protein)	Artificially synthesized sequence

3 CONTENT

eSens Streptococcus agalactiae QT PCR kit (ES3809A) includes:

Reagent	Description	Volume, ml	Quantity	
PCR-mix-1-FL <i>Streptococcus agalactiae</i>	clear liquid from colorless to light lilac colour	1.2	1 tube	
PCR-mix-2-FRT	colourless clear liquid	0.6	1 tube	
Polymerase (TaqF)	colourless clear liquid	0.06	1 tube	
TE-buffer	colourless clear liquid	0.5	1 tube	
DNA calibrators	K1 SAG	colourless clear liquid	0.2	1 tube
	K2 SAG	colourless clear liquid	0.2	1 tube
Positive Control DNA <i>Streptococcus agalactiae</i> and human DNA*	colourless clear liquid	0.1	1 tube	
Negative Control (C-)**	colourless clear liquid	1.2	2 tubes	
Internal Control STI-87 (IC)***	colourless clear liquid	0.6	2 tubes	

* must be used in the extraction procedure as Positive Control of Extraction.

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

*** add **10 μ l** of **Internal Control STI-87 (IC)** during the DNA extraction procedure directly to the sample/lysis mixture.

eSens Streptococcus agalactiae QT PCR kit is intended for 110 reactions (including controls and calibrators).

4 ADDITIONAL REQUIREMENTS

- DNA extraction kit or DNA extraction automatic station.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free 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 PCR tubes:
 - 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 with the temperature range from 2 to 8 °C.
- Deep-freezer with the temperature range 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 Streptococcus agalactiae QT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (blood plasma, cerebrospinal fluid (CSF), oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs)).

Pretreatment

6.1 Blood plasma

Tubes filled with whole blood are placed into the centrifuge at **800 g** for **10 min** at room temperature. Collect at least 1 ml of blood plasma from each sample with individual pipette tips with aerosol filters, and transfer the plasma into sterile 1.5-2.0 ml tubes. Centrifuge the tubes containing 1 ml of blood plasma for further 10-20 min at 11,000 rpm. For extraction use the sediment and 100 µl of over-sedimentary liquid.

7 WORKING CONDITIONS

eSens Streptococcus agalactiae QT 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-100-CE)

- For the automatic extraction

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

NOTE: Extract the DNA according to the manufacturer's protocol.
The DNA extraction for each sample is carried out in the presence of **Internal Control STI-87 (IC)**.

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 the **DNA** sample is **10 µl**.

NOTE: Tube selection depends on the thermocycler with "real-time" detection system being used.

1. Prior to the experiment prepare a mix of **PCR-mix-2-FRT** and **Polymerase (TaqF)**. Transfer the **Polymerase (TaqF)** tube contents (**60 µl**) to the **PCR-mix-2-FRT** tube (**600 µl**) and gently vortex avoiding the formation of foam.

NOTE: Prepared mix is intended for 120 samples. Store the samples at a temperature range of 2 to 8 °C for 3 months, to be used upon the need. If the prepared mix cannot be consumed in the course of 3 months, then prepare the mix for smaller number of reactions, for example, add 150 µl of PCR-mix-2-FRT and 15 µl of Polymerase (TaqF) (prepared mix is intended for 30 reactions)

2. Prepare the reaction mix. Take into account that in order to test one DNA sample in qualitative format, two controls of PCR amplification must be set up – Positive Control (**K2 SAG** DNA calibrator) and Negative Control (**TE-buffer**); and in quantitative format, five controls of PCR amplification must be set up: two DNA calibrators (**K1 SAG** and **K2 SAG**) with two repeats and Negative Control of PCR (**TE-buffer**). It is necessary to take reagents in reserve and calculate the volumes including 1 extra reaction.
3. In a separate tube mix **PCR-mix-1-FL *Streptococcus agalactiae*** with the previously prepared mix of **PCR-mix-2-FRT** and **Polymerase (TaqF)**. Calculation is based on the fact that each PCR loading includes:

10 µl of **PCR-mix-1-FL *Streptococcus agalactiae***
5 µl of **PCR-mix-2-FRT** and **Polymerase (TaqF)** mix

To make calculations for the required number of reactions, including testing of analysed and control samples, see Table 2.

Table 2

Scheme of preparation reaction mixture

Reagent volume for specified number of reactions			
Reagent volume per one reaction, µl		10,0	5,0
Number of clinical samples		PCR-mix-1-FRT <i>Streptococcus agalactiae</i> *	Mix of PCR-mix-2-FRT and Polymerase (TaqF)
for qualitative analysis	for quantitative analysis		
1	4	70	35
2	5	80	40
3	6	90	45
4	7	100	50
5	8	110	55
6	9	120	60
7	10	130	65
8	11	140	70
9	12	150	75
10	13	160	80
11	14	170	85
12	15	180	90
13	16	190	95
14	17	200	100
15	18	210	105
16	19	220	110
17	20	230	115
18	21	240	120
19	22	250	125
20	23	260	130
21	24	270	135
22	25	280	140
23	26	290	145
24	27	300	150
25	28	310	155
30	33	360	180

* Values are given including the reserve (volume calculation for 1 extra reaction) and including five controls (2 DNA calibrators K1 SAG and K2 SAG (both repeated) for quantitative analysis of *Streptococcus agalactiae* DNA and two controls (positive and negative controls) for qualitative analysis of *Streptococcus agalactiae* DNA.

NOTE: In simultaneous analysis of 120 samples, a simplified mix preparation scheme can be used: transfer all contents from one PCR-mix-2-FRT tube and all contents from one **Polymerase (TaqF)** tube to the **PCR-mix-1-FL *Streptococcus agalactiae*** tube.

4. Select the required number of tubes for amplification of the analyzed and control DNA samples.
5. Add **15 µl** of the reaction mix to each tube.

NOTE: Pipette tips with aerosol filters are used for transfer of DNA and control samples.

6. Add **10 µl** of **DNA samples** extracted from test and control samples into the prepared tubes.

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

7. Carry out the control amplification reactions:

For qualitative analysis:

NCA – Add **10 µl** of **TE-buffer** to the tube labelled NCA.

C+ – Add **10 µl** of **K2 SAG DNA calibrator** to the tube labelled C+

For quantitative analysis:

NCA – Add **10 µl** of **TE-buffer** to the tube labelled NCA.

K1 SAG – Add **10 µl** of **K1 SAG DNA calibrator** to two tubes

K2 SAG – Add **10 µl** of **K2 SAG DNA calibrator** to two tubes

8.2.2 Amplification

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

Table 3

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
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 Fluorescence detection		60	30 s Fluorescence detection	
	72	15 s		72	15 s	

2. Adjust the fluorescence channel sensitivity according to the *Technical Sheet*.
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.

9 DATA ANALYSIS

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

- channel for the **FAM** fluorophore registers the signal testifying the accumulation of the β -globin gene DNA (**IC Glob**) amplification product;
- channel for the **JOE** fluorophore registers the signal testifying the accumulation of ***Streptococcus agalactiae*** DNA amplification product;
- channel for **ROX** fluorophore registers a signal testifying the accumulation of amplification product of **Internal Control STI-87 (IC)** DNA.

Upon DNA extraction from oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs, results for two channels are considered: the channel for the **FAM** fluorophore registers a signal testifying the accumulation of amplification product for the β -globin gene DNA (**IC Glob**), and the channel for the **JOE** fluorophore - ***Streptococcus agalactiae*** DNA.

Upon DNA extraction from blood plasma and cerebrospinal fluid (CSF), results for two channels are considered: the channel for the **JOE** fluorophore - ***Streptococcus agalactiae*** DNA, the channel for the

ROX fluorophore registers the signal testifying the accumulation of amplification product of the **Internal Control STI-87 (IC)** DNA.

Results are interpreted by the presence (or absence) of an intercept between the fluorescence curve and 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 table.

Principle of interpretation of results for the DNA extraction from blood plasma and cerebrospinal fluid (CSF) is the following:

- *Streptococcus agalactiae* DNA is **detected** if the *Ct* value determined in the results table in the channel for the **JOE** fluorophore is less than the boundary *Ct* value specified in the *Technical Sheet*. Moreover, the fluorescence curve of the sample should intercept the threshold line in the area of characteristic exponential increase of fluorescence intensity.
- *Streptococcus agalactiae* DNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the channel for **JOE** fluorophore (fluorescence curve does not intercept the threshold line), and in the results table for the channel for the **ROX** fluorophore the *Ct* value is determined as less than the boundary *Ct* value specified in the *Technical Sheet*.
- The result is **invalid** if the *Ct* value is not determined (absent) for the given sample in the channel for **JOE** fluorophore, whereas the *Ct* value in the channel for the **ROX** fluorophore is not determined (absent) or is greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated for the corresponding clinical sample.
- For the clinical samples in which the *Ct* values in the channel for the **JOE** fluorophore are determined as greater than the boundary *Ct* value specified in the *Technical Sheet* the results are considered **equivocal**. In such cases, the PCR analysis should be repeated for the given sample with two repeats. In case of obtaining a reproducible positive *Ct* value, the result is interpreted as positive. If the results obtained are not reproducible, the result is interpreted as **equivocal**.

NOTE: Boundary *Ct* values are specified in the *Technical Sheet*.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of extraction and of amplification are correct (see Table 4 and Table 5). For quantitative analysis the results for Positive Control of Extraction (PCE) must be within the concentrations range outline in the *Technical Sheet* enclosed to the PCR kit.

Table 4

Results for controls of different stages of PCR analysis for DNA extraction from blood plasma and cerebrospinal fluid (CSF)

Control	Stage for control	Amplification results in the channel for fluorophore			
		JOE		ROX	
		Qualitative format	Qualitative format	Qualitative format	Qualitative format
C-	DNA extraction	Absent	Absent	< boundary value	< boundary value
PCE	DNA extraction	< boundary value	Obtained value is within the range defined in the <i>Technical Sheet</i>	< boundary value	< boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	<boundary value	–	<boundary value	–
K1 SAG K2 SAG	PCR	–	Ct value and calculated concentrations are defined	–	Ct value and calculated concentrations are defined

Principle of interpretation of results for the DNA extraction from oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs is the following:

- *Streptococcus agalactiae* DNA is **detected** if the Ct value determined in the results table in the channel for the **JOE** fluorophore is less than the boundary Ct value specified in the *Technical Sheet*. Moreover, the fluorescence curve of the given sample must intercept the threshold line in the area of characteristic exponential increase of fluorescence intensity.
- *Streptococcus agalactiae* DNA is **not detected** in a sample if the Ct value is not determined (absent) in the channel for **JOE** fluorophore (fluorescence curve does not intercept the threshold line), and in the results table for the channel for the **FAM** fluorophore for qualitative format the Ct value is determined as less than the boundary Ct value specified in the *Technical Sheet*, for quantitative format – the number of human genome equivalents (**IC Glob**) for the reaction is above **500**.
- The result is **invalid** if the Ct value is not determined (absent) for the given sample in the channel for **JOE** fluorophore, and in the channel for the **FAM** fluorophore for qualitative format the Ct value is greater than the boundary Ct value specified in the *Technical Sheet*, and for quantitative format – the number of human genome equivalents (**IC Glob**) for the reaction is above **500**. In such cases, the PCR analysis should be repeated for the corresponding clinical sample starting from the DNA extraction stage.
- For the clinical samples in which the Ct values in the channel for the **JOE** fluorophore are determined as greater than the boundary Ct value specified in the *Technical Sheet* the results

are considered **equivocal**. In such cases, the PCR analysis should be repeated for the given sample with two repeats. In case of obtaining the reproducible positive Ct value, the result is interpreted as positive. If the results obtained are not reproducible, the result is interpreted as **equivocal**.

Table 5

Results for controls of different stages of PCR analysis for DNA extraction from oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs

Control	Stage for control	Amplification results in the channel for fluorophore			
		JOE		FAM	
		Qualitative format	Quantitative format	Qualitative format	Quantitative format
C-	DNA extraction	Absent	Absent	> boundary value	> boundary value
PCE	DNA extraction	< boundary value	Obtained value is within the range defined in the <i>Technical Sheet</i>	< boundary value	< boundary value
NCA	PCR	Absent	Absent	> boundary value	> boundary value
C+	PCR	<boundary value	-	<boundary value	-
K1 SAG K2 SAG	PCR	-	Ct value and calculated concentration are defined	-	Ct value and calculated concentration are defined

For quantitative analysis the calculation of *Streptococcus agalactiae* DNA concentration in one ml of the sample upon extraction from 100 ml should use the following formula:

Calculated *Streptococcus agalactiae* DNA concentration x 100 = copies/ml.

10 TROUBLESHOOTING

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

1. If the result for a sample is invalid, PCR analysis needs to be repeated for the corresponding clinical sample.
2. If the positive signal is absent in the DNA calibrators, it may suggest that the amplification program is chosen wrongly and that other mistakes were made at the loading of PCR stage. In such case, repeat the PCR analysis for all samples.
3. If in the Negative Control of Extraction (C-) in the channel for the JOE fluorophores or in the Negative Control of Amplification (NCA) in the channels for the JOE and/or ROX fluorophores, the positive signal is detected, then the reagents and the samples must have been contaminated. In this case, repeat the analysis of the positive probes starting with the extraction stage, and undertake the necessary actions to identify the source of contamination.
4. If for the tested sample a positive signal is detected, but the fluorescence curve is missing the region of characteristic exponential increase (the curve represents a more of a straight line), that

may suggest that the threshold level or the basal line calculation parameters were incorrectly set up. Such result should not be interpreted as positive. If the result is obtained with the correctly set threshold level, it is necessary to repeat the PCR analysis for that sample.

11 TRANSPORTATION

eSens Streptococcus agalactiae QT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens Streptococcus agalactiae QT PCR kit** are to be stored at 2–8 °C when not in use. All components of the **eSens Streptococcus agalactiae QT 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: PCR-mix-1-FL *Streptococcus agalactiae*, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at the temperature range from minus 24 to minus 16 °C

NOTE: PCR-mix-1-FL *Streptococcus agalactiae* is to be kept away from light

13 SPECIFICATIONS

13.1 Sensitivity

Clinical material	DNA extraction kit	Amplification and detection kit	Analytical sensitivity, copies/ml	Linear measuring range, copies/ml
Blood plasma, cerebrospinal fluid (CSF), oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs	RIBO-prep	ES3809A	3x10 ²	1000-10,000,000

13.2 Specificity

The analytical specificity of **eSens Streptococcus agalactiae QT 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.

The reagents kit detects a fragment of *Streptococcus agalactiae* DNA. The specific activity of the reagents kit was confirmed by the analysis of *Streptococcus agalactiae* bacterial strains, and also by the analysis of clinical material with the followed confirmation of results using the methods of sequencing the amplification fragments.

The absence of kit components activity is shown with respect to the DNA of other bacterial pathogens (*Streptococcus pyogenes*, *Staphylococcus aureus*, *Neisseria meningitidis*, *Haemophilus parainfluenzae*, *Listeria monocitogenes*, *Klebsiella pneumonia*, etc.).

The clinical specificity of **eSens Streptococcus agalactiae QT 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		Contains sufficient for <n> tests
 LOT	Batch code		Use-by Date
 IVD	<i>In vitro</i> diagnostic medical device		Consult instructions for use
 VER	Version		Keep away from sunlight
	Temperature limit	NCA	Negative control of amplification
	Manufacturer	C-	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
 EC REP	Authorized representative in the European Community	IC	Internal control
	Caution	PCE	Positive control of extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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