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For Professional Use Only

eSens Helicobacter pylori QL PCR kit

REF ES3501B

Instructions for Use

1 INTENDED USE

eSens Helicobacter pylori QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Helicobacter pylori* DNA in the biological material (tissue (biopsy) material of gastric mucosa, feces, saliva), using real-time hybridization-fluorescence detection of amplified products. The material for PCR is DNA samples extracted from test material.

Indications and contra-indications for use of the reagent kit

The reagent kit is used in clinical laboratory diagnostics for the analysis of biological material taken from the persons with suspected helicobacteriosis without distinction of form and presence of manifestation.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

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

2 PRINCIPLE OF PCR DETECTION

Principle of testing is based on the DNA extraction from the samples of test material with the exogenous internal control sample (Internal Control-FL (IC)) and simultaneous amplification of DNA fragments of the detected microorganism (*Helicobacter pylori*) and DNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

Amplification of DNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA samples obtained at the extraction stage. 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 Helicobacter pylori QL PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically 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.

eSens Helicobacter pylori QL PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP).

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

Table 1

Channel for fluorophore	**FAM**	**JOE**
DNA-target	IC DNA	*Helicobacter pylori* DNA
Target gene	Artificially synthesized sequence	16S rDNA

3 CONTENT

eSens Helicobacter pylori QL PCR kit (ES3501B) includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-FL *Helicobacter pylori*	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-B	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
C+ *Helicobacter pylori*	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	0.5	1 tube
Buffer for elution A***	colorless clear liquid	1.2	3 tubes

* 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

*** the reagent is used instead of the reagent for elution of the nucleic acid extraction kit.

eSens Helicobacter pylori QL PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Transport medium for swabs.
- Reagent for pretreatment of viscous fluids (saliva).

- 0.9 % of sodium chloride (sterile saline solution) or phosphate buffered saline (PBS) (137 mM sodium chloride; 2,7 mM potassium chloride; 10 mM sodium monophosphate; 2 mM potassium diphosphate; pH=7,5±0,2).
- Glycerin for long storage of biological material (feces) in conditions of low-temperature freeze.
- Plastic container (50-60 ml) for sampling, storage and transportation of biological samples.
- Disposable screwed or tightly closed polypropylene 1.5-ml and/or 2.0-ml tubes for sampling and pretreatment.
- Teflon-tipped pestle for homogenization in 1.5-ml tubes.
- Sterile pipette tips (up to 1,000 µl) and sterile pipette tips with aerosol filters (up to 100 µl, 200 µl and 1,000 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir to throw off and inactivate the material.
- Disposable powder-free gloves and a laboratory coat.

For DNA extraction and amplification

- DNA extraction kit.
- Sterile pipette tips with aerosol filters (up to 10 µl, 100 µl and 200 µl).
- Tube racks.
- Vortex mixer.
- 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:
    - screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
    - thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent 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.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- 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 Helicobacter pylori QL PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (tissue (biopsy) material of gastric mucosa, feces, saliva).

Sampling

Tissue (biopsy) material of gastric mucosa. Tissue material (pieces of tissue no more than 5 mm in diameter) is placed into microtubes with screw caps or into 1.5-2.0-ml tubes with snap caps containing 0.1-0.3 ml of transport medium.

The tissue (biopsy) material of gastric mucosa can be stored before pretreatment:

- at the temperature from 18 to 25 °C – for 6 hours;
- at the temperature from 2 to 8 °C – for 3 days;
- at the temperature from minus 24 to minus 16 °C – for 1 week;
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

The pre-frozen material can be transported at 2–8 °C for 1 day.

Feces are taken from a disposable reservoir (for example, a petrie dish, disposable plastic bag) placed into a bed-pan or disposable diapers (for younger children). When using a disposable diaper for

children with liquid stool, place a cotton pad into the diaper before the use for obtaining the sufficient quantity of sample.

NOTE: It is forbidden to take feces samples directly from a bed-pan or another reservoir for multiple use (without distinction of disinfection methods).

Using a separate filter tip or disposable spatula transfer about 1.0 g (or 1.0 ml) of the sample into special disposable plastic container.

The feces samples can be stored before pretreatment:

- at the temperature from 18 to 25 °C – for 6 hours;
- at the temperature from 2 to 8 °C – for 3 days;
- at the temperature from minus 24 to minus 16 °C – for 1 week;
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is required.

The feces samples can be transported at 2–8 °C for 3 days.

Saliva should be obtained after threefold rinsing the oral cavity with saline solution or boiled water. Saliva is taken into sterile dry disposable 2.0-ml tubes in an amount not less than 0.2-1.0 ml. Close the tube tightly with a cap, avoiding gaps and crumpling of the inner part of the cap. Mark the tube.

The saliva samples can be stored before pretreatment:

- at the temperature from 18 to 25 °C – for 6 hours;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 week;
- at the temperature not more than minus 68 °C – for a long time. Only one freeze-thaw cycle is allowed.

Pretreatment

Tissue (biopsy) material of gastric mucosa is to be pretreated.

Homogenize a sample of tissue (biopsy) material of gastric mucosa in a tube with transport medium by rubbing it against the walls of the tube using a teflon pestle (new for each sample). A short centrifugation should be performed after the procedure to sediment the drops from the walls of the tube.

The pretreated samples of tissue (biopsy) material of gastric mucosa can be stored before PCR-analysis:

- at the temperature from 18 to 25 °C – for 6 hours;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 week;
- at the temperature not more than minus 68 °C – for a long time.

Feces are to be pretreated. Fecal suspension preparation:

1. Take the required number of disposable 1.5-ml tubes respectively to the number of samples. Add 1.0 ml of PBS into each tube (use 15-20 % solution of glycerin in PBS when necessary to store the suspension more than 1 day under refrigeration).
2. Using a new one filter tip (or disposable spatula) for each sample add 0.1 g (0.1 ml) of feces into each tube and resuspend thoroughly on vortex due to obtain homogenous suspension. Optimal concentration of suspension is ~ 10 % (by the pellet volume after centrifugation).

Sediment the drops from the tube caps by short centrifugation on vortex (no more than 10 sec).

Liquid semitransparent feces are used for express filtration without previous obtaining the suspension.

Express filtration of fecal suspension (for viral and bacterial pathogens detection):

1. For express filtration use two tips up to 1.0 ml (with filter and without filter) and a cut lower part of cotton probe (cotton bud).
2. Put the cut lower part of disposable cotton probe (cotton bud) in the tip without aerosol filter and fix it by pushing into the necked part of the tip.
3. Take 1.0 ml of fecal suspension by the filter tip, put it in the prepared tip with cotton filter and carry out the pressing-filtration into a new disposable tube. In case of difficult filtration it is recommended to decrease the fecal suspension concentration.
4. 100 µl of filtrate is used for DNA extraction.

The pretreated samples of feces suspension can be stored before PCR-analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is required.

The samples of fecal suspension can be transported at 2–8 °C for 1 day.

Saliva is to be pretreated.

If mucus is present in the sample, Mucolysin reagent should be added to the sample. In order to reduce the viscosity, a threefold amount of Mucolysin reagent respectively to the amount of saliva should be added to the container with saliva and incubated at the room temperature (from 18 to 25 °C), mixing occasionally for 10-20 min (until visual clarification). 100 µl of thin saliva is used for DNA extraction.

The pretreated thin saliva can be stored before PCR-analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C – for a long time. Only one freeze-thawing cycle is required.

Interfering substances and limitations of using test material samples

Feces samples taken directly from a bed-pan or another reservoir for multiple use (without distinction of disinfection methods) are inapplicable for analysis.

In order to control the DNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control-FL (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material (tissue (biopsy) material of gastric mucosa, feces, saliva) used for the study were selected to assess potential interference.

Model samples of biological material without adding and with the addition of potential interfering substances were tested. The concentration of each potential interfering substance is specified in Table 2. Model samples contained quality control sample (QCS) with *Helicobacter pylori* DNA concentration of $1x10^3$ GE/ml.

Table 2

Test material	**Type of potential interferent**	**Potential interferent**	**Tested concentration in a sample**	**Interference presence**
Tissue (biopsy) material of gastric mucosa	Endogenous substances	Whole blood	Up to 40 %	Not detected
	Exogenous substances	Formalin 10% neutral	Up to 10 %	Not detected
		Clarithromycin, 250 mg capsules + Flemoxin Solutab (Amoxicillin 1,000 mg)	Up to 10 mg/ml Clarithromycin + 20 mg/ml Amoxicillin	Not detected
		De-nol (bismuth tripotassium dicitrate 304.6 mg (in terms of bismuth oxide 120 mg))	Up to 12.2 mg/ml bismuth tripotassium dicitrate (or 4.8 mg/ml in terms of bismuth oxide)	Not detected
		Omeprazole-Akrikhin, enterosoluble capsules, omeprazole 20 mg	Up to 1.2 mg/ml	Not detected
Feces	Endogenous substances	Whole blood	Up to 40 %	Not detected
		Fecal fats	Up to 40 %	Not detected
		Mucin (mucus)	Up to 3 %	Detected at concentration greater than 10 mg/ml
	Exogenous substances	'Enterofuril' oral suspension	Up to 4.25 mg/ml	Not detected
		'Enterosgel', oral paste	Up to 174.75 mg/ml	Not detected
		Dextrin (Russia)	Up to 68.6 mg/ml	Not detected
		Whole blood	Up to 10 %	Not detected

Saliva	Endogenous substances	Mucin (mucus)	Up to 1 %	Detected at concentration greater than 1.0 mg/ml
	Exogenous substances	Miramistin 0,01 %	Up to 10 %	Not detected
		Chlorhexidine 0,05 %	Up to 10 %	Not detected

7 WORKING CONDITIONS

eSens Helicobacter pylori QL PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

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;
  - RIBO-prep.

- For the automatic extraction

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

NOTE: Extract the DNA according to the manufacturer's protocol.

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

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:

   - 10 µl of PCR-mix-FL Helicobacter pylori,
   - 5 µl of PCR-buffer-B,
   - 0.5 µl of Polymerase (TaqF).

   Prepare the reaction mixture for the total number of test and control samples plus some extra reaction. See numbers of control samples in item 7.

The calculation for the required number of reactions including testing the test and control samples can be performed according to Table 3.

Table 3

Reaction mixture preparation scheme

		Reagent volume for specified number of reactions		
Reagent volume per one reaction, µl		**10.0**	**5.0**	**0.5**
Number of test samples	**Number of Reactions***	**PCR-mix-FL *Helicobacter pylori***	**PCR-buffer-B**	**Polymerase (TaqF)**
2	**6**	**60**	**30**	**3.0**
4	**8**	**80**	**40**	**4.0**
6	**10**	**100**	**50**	**5.0**
8	**12**	**120**	**60**	**6.0**
10	**14**	**140**	**70**	**7.0**
12	**16**	**160**	**80**	**8.0**
14	**18**	**180**	**90**	**9.0**
16	**20**	**200**	**100**	**10.0**
18	**22**	**220**	**110**	**11.0**
20	**24**	**240**	**120**	**12.0**
22	**26**	**260**	**130**	**13.0**
24	**28**	**280**	**140**	**14.0**
26	**30**	**300**	**150**	**15.0**
28	**32**	**320**	**160**	**16.0**

* Number of reaction including the number of test samples (N), the controls of extraction stage and PCR, and one extra reaction (N+1+2+1).

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tube with **PCR-mix-FL *Helicobacter pylori*.** Thoroughly vortex all the reagents of the PCR kit and sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of **PCR-mix-FL *Helicobacter pylori*, PCR-buffer-B** and **polymerase (TaqF)**. Sediment the drops by vortex.
4. Take the required number of the tubes or strips for PCR of DNA of test and control samples.
5. Transfer **15 µl** of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. **Add 10 µl** of **DNA samples** obtained by extraction of test samples.

NOTE: Avoid transferring the sorbent together with the DNA samples extracted with the reagent kit for extraction on silica gel.

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 **C+ *Helicobacter pylori*** 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).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

8.2.2 Amplification

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

Table 4

Amplification program for rotor-type* and plate-type instruments**

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	**50**	**30 min**	**–**	**1**
2	**95**	**15 min**	**–**	**1**
3	**95**	**10 s**	**–**	**45**
	60	**25 s**	**FAM, JOE**	
	72	**10 s**	**–**	

* For example, Rotor-Gene Q (QIAGEN, Germany).

** For example, CFX 96 (Bio-Rad, USA).

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores.

NOTE: The given program (Table 4) can be used for all eSens PCR kits, intended for detection and differentiation of DNA/RNA of microorganisms causing acute intestinal infections, with the possibility of their combined use in one run of the instrument. If only the tests for DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 30 min) can be omitted for time saving. If other tests are carried out simultaneously, the detection is also enabled in other used channels.

2. Adjust the fluorescence channel sensitivity.
3. Insert tubes into the reaction module of the device.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation before placing them into the instrument.

Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

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 3000/6000

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	10%

Note - If the fluorescence curves in the JOE/Yellow channel do not correspond to the exponential growth, then *NTC threshold value* can be increased up to 15 %.

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 JOE/HEX	Set the threshold line in the logarithmic scale at the level where fluorescence curves are linear and do not cross the curves of negative samples. The threshold line should be set at the level corresponding to 10-20 % of maximum fluorescence level obtained for any positive sample at 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 three channels:

Table 5

Channel for the fluorophore	FAM	JOE
Amplification product	IC DNA	*Helicobacter pylori* DNA

Results are interpreted by the crossing (or not-crossing) the S-shaped (sigmoid) 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.

Table 6

Results interpretation

***Ct* value in the channel for the fluorophore**		**Result**
FAM	**JOE**	
determined or absent	< boundary value	*Helicobacter pylori* DNA **is detected**
< boundary value	absent or > boundary value	*Helicobacter pylori* DNA **is NOT detected**
absent or > boundary value	absent or > boundary value	**Invalid*** result

* In case of **invalid** result, the PCR-analysis should be repeated for the corresponding test sample starting from the DNA extraction stage.

Table 7

Results for controls

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

Interpretation of some test samples is not possible if the results for the controls deviate from the results specified above (see *10.Troubleshooting*).

Boundary *Ct* values

Sample	Rotor-type instrument		Plate-type instrument	
	Channel for fluorophore			
	FAM	JOE	FAM	JOE
C-	38	*Ct* value is absent or > 38	40	*Ct* value is absent or > 40
NCA	*Ct* value is absent or >38	*Ct* value is absent or > 38	*Ct* value is absent or > 40	*Ct* value is absent or > 40
C+	38	38	40	40
Test samples	38	38	40	40

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 any of the specified channels for fluorophores (see Table 7) is greater than the boundary value or absent. The results interpretation is not possible for samples in which *Helicobacter pylori* DNA was not detected, the PCR analysis should be repeated for these samples. For the samples in which *Helicobacter pylori* DNA was detected, it is necessary to follow the steps specified in point 4.
2. For the Negative Control of Extraction (C–):
    - The *Ct* value determined in the channel for the JOE fluorophore is less than the boundary value. The contamination of laboratory with amplification products or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. The results interpretation is not possible for the samples in which *Helicobacter pylori* DNA was detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for these samples;
    - The *Ct* value determined in the channel for the FAM fluorophore is absent or greater than the boundary value. The results interpretation for the test samples is carried out according to Table 7.
3. For the Negative Control of amplification (NCA):
    - The *Ct* value determined in the channel for the JOE fluorophore is less than the boundary value. The contamination of laboratory with amplification products or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. The results interpretation is not possible for the samples in which *Helicobacter pylori* DNA was detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for these samples;
    - The *Ct* value determined in the channel for the FAM fluorophore is less than the boundary value. The contamination of laboratory with amplification products or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. The results interpretation is not possible for the samples in which *Helicobacter pylori* DNA

was not detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for these samples.

4. If the *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence (in the "raw" data view mode) is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level. If the result has been obtained with the correct level of threshold line (base line), the amplification should be repeated for this sample.

11 TRANSPORTATION

eSens Helicobacter pylori 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 Helicobacter pylori QL PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-FL *Helicobacter pylori*, PCR-buffer-B and polymerase (TaqF).

All components of the **eSens Helicobacter pylori 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: PCR-mix-FL *Helicobacter pylori*, PCR-buffer-B and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C.

PCR-mix-FL *Helicobacter pylori* is to be kept away from light.

PCR-mix *Helicobacter pylori*-Lyo is to be kept in packages with a desiccant away from light.

13 SPECIFICATIONS

13.1 Analytical sensitivity (limit of detection)

Table 8

Test material	**Nucleic acid extraction kit**	**PCR kit**	**Analytical sensitivity (limit of detection), copies/ml**
Tissue (biopsy) material of gastric mucosa, feces, saliva	DNA-sorb-B, RIBO-prep, ePure Bacterial DNA Extraction Kit	eSens Helicobacter pylori QL PCR kit	$1x10^3$

13.2 Specificity

The analytical specificity of **eSens *Helicobacter pylori 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.

The PCR kit detects *Helicobacter pylori* DNA fragments. The analytical specificity was proved when investigating the DNA of the following microorganisms/strains:

1. Strains from ATCC (American Type Culture Collection, USA) in concentration no less than $1x10^6$ GE/ml:

- *Helicobacter pylori* Strain J99 (ATCC® 700824™) and NCTC 11637 (ATCC® 43504™);
- *Campylobacter jejuni* subsp. *jejuni* (ATCC® 33560™), *Campylobacter coli* (ATCC® 49941™), *Campylobacter fetus* subsp. *fetus* (ATCC® 27374™), *Campylobacter hominis* (ATCC® BAA-381™);
- *Acinetobacter baumannii* (ATCC® 19606™), *Bacteroides fragilis* (ATCC® 25285™), *Bordetella bronchiseptica* (ATCC® 10580™), *Bordetella bronchiseptica* (ATCC® 4617™), *Bordetella pertussis* (ATCC® 9340™), *Candida albicans* (ATCC® 14053™), *Candida guilliermondii* (ATCC® 6260™), *Candida krusei* (ATCC® 14243™), *Clostridium difficile* (ATCC® 9689™), *Clostridium septicum* (ATCC® 12464™), *Corynebacterium jeikeium* (ATCC® 43734™), *Corynebacterium minutissimum* ATCC® 23348™, *Corynebacterium xerosis* (ATCC® 373™), *Eggerthella lenta (Eubacterium lentum)* (ATCC® 43055™), *Enterobacter aerogenes* (ATCC® 13048™), *Enterobacter cloacae* (ATCC® 13047™), *Enterococcus faecalis* (ATCC® 29212™), *Enterococcus faecalis* (*vancomycin resistant*) (ATCC® 51299™), *Enterococcus faecium* (ATCC® 35667™), *Erysipelothrix rhusiopathiae* (ATCC® 19414™), *Escherichia coli* (ATCC® 25922™), *Escherichia coli* (ATCC® 35218™), *Gardnerella vaginalis* (ATCC® 14018™), *Haemophilus influenzae* (ATCC® 33930™), *Haemophilus influenzae* (ATCC® 9006™), *Haemophilus influenzae* (ATCC® 10211™), *Haemophilus parainfluenzae* (ATCC® 7901™), *Klebsiella oxytoca* (ATCC® 49131™), *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 27736™), *Listeria grayi* (*murrayi*) (ATCC® 25401™), *Listeria innocua* (ATCC® 33090™), *Listeria monocytogenes* (ATCC® 7644™), *Moraxella* (*Branhamella*) *catarrhalis* (ATCC® 25238™), *Moraxella* (*Branhamella*) *catarrhalis* (ATCC® 8176™), *Moraxella catarrhalis* (ATCC® 25240™), *Neisseria meningitidis* (ATCC® 13102™), *Neisseria meningitidis* (ATCC® 13090™), *Neisseria lactamica* (ATCC® 23970™), *Neisseria gonorrhoeae* (ATCC® 19424™), *Neisseria gonorrhoeae* (ATCC® 49926™), *Proteus mirabilis* (ATCC® 12453™), *Proteus vulgaris* (ATCC® 6380™), *Propionibacterium acnes* (ATCC® 11827™), *Pseudomonas aeruginosa* (ATCC® 15442™), *Rhodococcus equi* (ATCC® 6939™), *Salmonella enterica* subsp. *enterica serovar Typhimurium* (ATCC® 14028™), *Serratia marcescens* (ATCC® 14756™), *Staphylococcus aureus* subsp. *aureus* (ATCC® 6538P™), *Staphylococcus aureus* subsp. *aureus* (MRSA) (ATCC® 43300™), *Staphylococcus aureus* subsp. *aureus* (ATCC® 29213™), *Staphylococcus aureus* subsp. *aureus* (ATCC® 25923™), *Staphylococcus aureus* subsp. *aureus* (ATCC® 33862™), *Staphylococcus aureus* subsp. *aureus* (MRSA) (ATCC® 33591™), *Staphylococcus aureus* subsp. *aureus* (ATCC® 12600™), *Staphylococcus epidermidis* (ATCC® 12228™), *Staphylococcus haemolyticus* (ATCC® 29970™), *Staphylococcus saprophyticus* (ATCC® 49907™), *Stenotrophomonas maltophilia* (ATCC® 13637™), *Streptococcus agalactiae* (ATCC® 12386™), *Streptococcus agalactiae* (ATCC® 13813™), *Streptococcus dysgalactiae* subsp. *equisimilis* (ATCC® 12388™), *Streptococcus equi* subsp. *equi* (ATCC® 9528™), *Streptococcus bovis* (Group D) (ATCC® 9809™), *Streptococcus mutans* (ATCC® 35668™), *Streptococcus pneumoniae* (ATCC® 49619™), *Streptococcus pneumoniae* (ATCC® 6303™), *Streptococcus pneumoniae* (ATCC® 27336™), *Streptococcus pneumoniae* (ATCC® 6305™), *Streptococcus pyogenes* (ATCC® 19615™), *Streptococcus salivarius* (ATCC® 13419™), *Streptococcus uberis* (ATCC® 700407™), *Vibrio parahaemolyticus* (ATCC® 17802™), *Vibrio vulnificus* (ATCC® 27562™).

2. Human DNA in concentration of 0.2 mg/ml.

The nonspecific reactions were not observed while testing the DNA samples of the above mentioned organisms, as well as human DNA.

The clinical specificity of **eSens Helicobacter pylori QL PCR kit** was confirmed in laboratory clinical trials.

The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3 Repeatability and reproducibility

Repeatability and reproducibility were determined by testing positive and negative model samples. Positive samples were a quality control sample (QCS) containing ***Helicobacter pylori*** DNA with concentration of $1x10^3$ GE/ml, Negative Control (C–) was used as a negative sample.

Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing different lots of PCR kit in different laboratories, by different operators, on different days, using different equipment. The results are presented in Table 9.

Table 9

Sample type	Repeatability		Reproducibility	
	Number of samples	Agreement of results, %	Number of samples	Agreement of results, %
Positive	10	100	40	100
Negative	10	100	40	100

13.4 Diagnostic characteristics

The following samples were used for evaluation of the diagnostic characteristics of the PCR kit:

- 115 gastric mucosal biopsy samples obtained from patients with suspected *Helicobacter pylori* infection in the period 2014-2021;
- 187 feces samples from patients with acute intestinal infections (from foci of group incidence and sporadic cases of intestinal infections) and from patients with suspected *Helicobacter pylori* infection. Feces samples negative for bacterial pathogens were obtained from patients with acute intestinal infections and clinically healthy individuals in the period 2018-2021;
- 145 saliva samples obtained from patients with suspected *Helicobacter pylori* infection in 2021 and clinically healthy individuals in the period 2018-2021;
- 44 model saliva samples contaminated with the bacterial pathogen (*Helicobacter pylori* DNA (*Helicobacter pylori* NCTC 11637 (ATCC® 43504™))).

Table 10

The results of testing eSens Helicobacter pylori QL PCR kit in comparison with the reference assay

Test material	The results of application of eSens Helicobacter pylori QL PCR kit		Results of using the reference assay	
			Positive	Negative
Tissue (biopsy) material of gastric mucosa	115 samples were tested	**Positive**	69	6
		Negative	0	40
Feces	187 samples were tested	**Positive**	53	5
		Negative	1	128
		Positive	53	2

Saliva	189 samples were tested	**Negative**	0	134

Table 11

Diagnostic characteristics of eSens Helicobacter pylori QL PCR kit

Test material	**Diagnostic sensitivity* with a confidence level of 95 %**	**Diagnostic specificity ** with a confidence level of 95 %**
Tissue (biopsy) material of gastric mucosa	100 (95.75-100) %	86.96 (76.96-96.96) %
Feces	98.15 (94.47-100) %	96.24 (92.98-99.50) %
Saliva	100 (94.5-100) %	98.5 (96.49-100) %

* Relative sensitivity in comparison with applied reference method.

** Relative specificity in comparison with applied reference method.

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	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limit		Keep dry
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C-	Negative control of extraction
		C+	Positive control of amplification
⚠	Caution	IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		
02_06/2025	9 DATA ANALYSIS	**Table 7** was changed.

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