



For *in Vitro* Diagnostic Use

TABLE OF CONTENTS

1. INTENDED USE.....	3
2. PRINCIPLE OF PCR DETECTION.....	3
3. CONTENT.....	3
4. ADDITIONAL REQUIREMENTS.....	3
5. GENERAL PRECAUTIONS.....	4
6. SAMPLING AND HANDLING.....	4
7. PROTOCOL.....	5
8. DATA ANALYSIS.....	6
9. TROUBLESHOOTING.....	7
10. STABILITY AND STORAGE.....	8
11. SPECIFICATIONS.....	8
12. REFERENCES.....	8
13. QUALITY CONTROL.....	8
14. EXPLANATION OF SYMBOLS.....	9

AmpliSens[®] *Helicobacter pylori*-EPh PCR kit

Instruction Manual

AmpliSens[®]

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1. INTENDED USE.

AmpliSens® *Helicobacter pylori*-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Helicobacter pylori* in the clinical material (biopsy sample of stomach mucous membrane) by using electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE OF PCR DETECTION.

Helicobacter pylori detection by the polymerase chain reaction (PCR) is based on the amplification of specific region of DNA of pathogen genome using special *Helicobacter pylori* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® *Helicobacter pylori*-EPh PCR kit** uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer. Wax melting and reaction mix components occur only at 95 °C.

3. CONTENT.

AmpliSens® *Helicobacter pylori*-EPh PCR kit is produced in 2 forms:

AmpliSens® *Helicobacter pylori*-EPh PCR kit variant 50 R (tubes 0.5 ml), **REF** B9-50-R0,5-CE.

AmpliSens® *Helicobacter pylori*-EPh PCR kit variant 50 R (tubes 0.2 ml), **REF** B9-50-R0,2-CE.

AmpliSens® *Helicobacter pylori*-EPh PCR kit variant 50 R:

Reagent	Description	variant 50 R	
		Volume (ml)	Amount
PCR-mix-1-R <i>Helicobacter pylori</i> ready-to-use single-dose test tubes (under wax)	colorless, clear liquid	0.005	55 tubes of 0.5 or 0.2 ml
PCR-mix-2 blue	clear liquid of blue color	0.6	1 tube
Mineral oil for PCR	colorless viscous liquid	2.0	1 dropper bottle
Positive Control DNA <i>Helicobacter pylori</i> (C+)	colorless, clear liquid	0.1	1 tube
TE-buffer	colorless, clear liquid	0.5	1 tube
Negative Control (C-)*	clear liquid of stramineous color	1.6	1 tube

* must be used in the isolation procedure as Negative Control of Extraction (see “DNA-sorb-B”, **REF** K1-2-50-CE protocol).

AmpliSens® *Helicobacter pylori*-EPh PCR kit variant 50 R is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Agarose gel detection kit.

- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- PCR box.
- Tube racks.
- Personal thermocyclers (for example, Palm-Cycler (Corbett Research, Australia), GeneAmp PCR System 2400, GeneAmp PCR System 2700 (Applied Biosystems, USA), Terzik (DNA-Technology, Russia), MiniCycler, PTC-100 (MJ Research, USA), Omn-E (ThermoHybaid)).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator with temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional, it should begin in the Extraction Area move to the

Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which 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.



Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [2]. It is recommended to read this handbook before starting work.

AmpliSens® *Helicobacter pylori*-EPh PCR kit is intended for analysis of DNA extracted with DNA isolation kits from:

- biopsy sample of stomach mucous membrane.

6.1. *Biopsy sample of stomach mucous membrane* should be placed in a saline solution (0.1 ml) and delivered within 1 day in a container with an icepack. Single biopsy sample can be used as well as a pool of 3-5 samples obtained from different sites of stomach mucous membrane. The sample should be thoroughly homogenized by using sterile porcelain mortar and a pestle in 0.15 M sodium chloride added in small portions (200 µl of solution per one biopsy specimen). Prepared suspension should be transferred in a tube. Use 100 µl for DNA extraction.



Only one freeze-thaw cycle of clinical material is allowed.

7. PROTOCOL.

7.1. DNA Isolation.

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-B", **REF** K1-2-50-CE.



Carry the DNA isolation in compliance with the manufacturer protocol.



Positive Control DNA *Helicobacter pylori* (C+) must be used during DNA isolation procedure. Add 10 µl of Positive control DNA *Helicobacter pylori* (C+) and 90 µl of Negative Control (C-) into the tube labeled PCE (Positive Control of Extraction).

7.2. Preparing the PCR.

Total reaction volume - **25 µl**, volume of DNA sample - **10 µl**.

7.2.1. Preparing tubes for PCR.

1. Prepare the required number of the PCR tubes with **PCR-mix-1-R *Helicobacter pylori*** for amplification of DNA from clinical and control samples.

2. Add **10 µl** of **PCR-mix-2 blue** to the surface of wax layer of each tube ensuring that it does not fall under the wax and mix with PCR-mix-1-R *Helicobacter pylori*.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl). When using thermocycler with heating cover this step could be omitted.
4. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples.
5. 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 *Helicobacter pylori*** to the tube labeled C+ (Positive Control of Amplification).

7.2.2 Amplification.

Run the following program on the thermocycler (see table 1). When the temperature reaches 95 °C (pause regimen), insert tubes to cells of amplifier and press button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before their insertion in thermocycler.

Table 1

Programming thermocyclers for *Helicobacter pylori* DNA amplification

Step	Thermocyclers with active temperature adjustment						Thermocyclers with block temperature adjustment		
	GeneAmp PCR System 2400 (Perkin Elmer), Omn-E (ThermoHybaid), Terzik (DNA-Technology)			GeneAmp PCR System 2700 (Applied Biosystems), Palm-Cycler (Corbett Research)			PTC-100, MiniCycler (MJ Research)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	10 sec	42	95 °C	10 sec	42	95 °C	1 min	42
	65 °C	10 sec		65 °C	25 sec		65 °C	1 min	
	72 °C	10 sec		72 °C	25 sec		72 °C	1 min	
3	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
4	10 °C	storage		10 °C	storage		10 °C	storage	

Amplification in thermocycler with block temperature adjustment lasts 2 h, in thermocycler with active temperature adjustment — 1 h 30 min.

After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2 – 8 °C (be sure to warm the samples to room temperature before running electrophoresis).

8. DATA ANALYSIS.

It's recommended to use the following detection agarose kit:

- “EPh” variant 200, **REF** K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7%). The length of specific amplified DNA fragments is:

- *Helicobacter pylori* - 520 bp



Put the protective mask or use the glass barrier while watching and photographing the gel.

8.1. Results interpretation.

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel 520 bp	Interpretation
PCE	DNA isolation	Yes	OK
C-	DNA isolation	No	OK
NCA	Amplification	No	OK
C+	Amplification	Yes	OK

- The sample is considered to be positive for *Helicobacter pylori* DNA if the band of 520 bp is present in agarose gel.
- The sample is considered to be negative for *Helicobacter pylori* DNA if the band of 520 bp is absent. Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

9. TROUBLESHOOTING.

Results of analysis are not being registered in the following cases:

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests are to be re-installed. Discard any reagents that may be suspect.
- If in lanes corresponding to positive control (PCE, C+) band of 520 nucleotide pairs is not observed, result of analysis is irrelevant. It can be caused by mistake in PCR conducting or amplification program fault.
- If in lines nonspecific bands at different levels are presented, it may be caused by lack of “hot start” or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C-) specific band of 520 bp appears it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

10. STABILITY AND STORAGE.

All components of **AmpliSens® *Helicobacter pylori*-EPh** PCR kit are to be stored at the temperature between 2 and 8 °C when not in use. All components of the PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® *Helicobacter pylori*-EPh** PCR kit is no less than 1x10³ genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens® *Helicobacter pylori*-EPh** PCR kit are guaranteed only when additional kits of reagents, “DNA-sorb-B” and “EPh” (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology), are used.

11.2. Specificity.

Specificity of **AmpliSens® *Helicobacter pylori*-EPh** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.












12. REFERENCES.

1. Colding H, Hartzen SH, Roshanisefat H, Andersen LP, Krogfelt KA. Molecular methods for typing of *Helicobacter pylori* and their applications. FEMS Immunol Med Microbiol. 1999 Jun; 24(2):193-9.
2. Manual “Sampling, transportation and storage of clinical material for PCR diagnostics”, developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers” Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL.

In compliance with Federal State Institution of Science Central Research Institute of Epidemiology ISO 13485 – certified Total Quality Management System, each lot of **AmpliSens® *Helicobacter pylori*-EPh** PCR kit is tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Contains sufficient for <N> tests
	Authorised representative in the European Community.		Consult instructions for use
	Caution, consult accompanying documents	NCA	Negative control of Amplification
C+	Positive control of Amplification	C-	Negative control of Extraction
PCE	Positive control of Extraction		