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For Professional Use Only

AmpliSens® *Helicobacter pylori*-EPh PCR kit

Instruction Manual

AmpliSens®

EC REP

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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* DNA in clinical material (gastric mucosa biopsy samples) by using electrophoretic detection of the amplified products in agarose gel.

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

2. PRINCIPLE OF PCR DETECTION

Helicobacter pylori DNA detection by the polymerase chain reaction (PCR) is based on the amplification of a specific region of the pathogen genome DNA using specific *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 the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer. Wax melts and reaction components mix 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 includes:

Reagent	***Description***	***Variant 50 R***	
		Volume (ml)	***Amount***
PCR-mix-1-R *Helicobacter pylori* 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	blue clear liquid	0.6	1 tube
Mineral oil for PCR	colorless viscous liquid	2.0	1 dropper bottle
Positive Control DNA *Helicobacter pylori* (C+$_{Helicobacter\ pylori}$)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	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, UK)).
- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °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 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 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 another suitable disinfectant.
- Avoid contact with the skin, eyes, and mucosa. If skin, eyes, and mucosa contact, immediately flush with water and seek medical attention.

- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- The laboratory process must be one-directional, it should begin in the Extraction Area and move to the Amplification and Detection Areas. 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 are 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:

- gastric mucosa biopsy samples.

6.1. *Gastric mucosa biopsy samples* should be placed in saline (0.1 ml) and delivered to the laboratory within 1 day in a container with ice. Single biopsy sample can be used as well as 3–5 pooled samples taken from different areas of gastric mucosa. The sample should be thoroughly homogenized in a sterile porcelain mortar with a pestle in 0.15 M NaCl added in small portions (200 µl of solution per one biopsy specimen). Thus prepared suspension should be transferred to a tube. Use 100 µl for DNA extraction.

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

7. WORKING CONDITIONS

AmpliSens® ***Helicobacter pylori*-EPh** PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Isolation

It is recommended to use the following nucleic acid extraction kits:

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

Extract DNA in compliance with the manufacturer's protocol.

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

8.2. Preparing PCR

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

8.2.1. 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 the 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** (~ 25 µl).
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+*Helicobacter pylori* - Add 10 µl of **Positive Control DNA *Helicobacter pylori*** to the tube labeled C+*Helicobacter pylori* (Positive Control of Amplification).

8.2.2 Amplification.

Run the following program in the thermocycler (see Table 1). When the temperature reaches 95 °C (pause mode), insert tubes into the cells of the thermocycler and press the button to continue.

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them in the thermocycler.

Table 1

Programming thermocyclers for *Helicobacter pylori* DNA amplification

	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)**		
Step	Tempe-rature	Time	Cycles	Tempe-rature	Time	Cycles	Tempe-rature	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 s	42	**95 °C**	10 s	42	**95 °C**	1 min	42
	65 °C	10 s		**65 °C**	25 s		**65 °C**	1 min	
	72 °C	10 s		**72 °C**	25 s		**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 thermocyclers with block temperature adjustment lasts for 2 h; in thermocyclers with active temperature adjustment, for 1 h 30 min.

After the reaction is completed, PCR tubes should be collected and transferred to the room for PCR product 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, and for a long time at –16 °C (warm up samples to room temperature before running electrophoresis).

9. DATA ANALYSIS

It is 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 on a protective mask or use a glass barrier while watching and photographing the gel.

9.1. 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+*Helicobacter pylori*	Amplification	Yes	OK

- The sample is considered to be positive for *Helicobacter pylori* DNA if the 520-bp band is present in agarose gel.
- The sample is considered to be negative for *Helicobacter pylori* DNA if the 520-bp band is absent.

In addition to the specific bands, fuzzy bands corresponding to primer dimers may appear in lanes below the 100-bp level.

10. TROUBLESHOOTING

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

- If results of analysis of control points do not correspond to those listed above (Table 2), the tests should be repeated. Discard any reagents that may be suspect.
- If the 520-bp band is absent in lanes corresponding to positive controls (PCE, C+*Helicobacter pylori*), the result of analysis is irrelevant. This may be caused by errors in PCR or incorrect amplification program.
- The appearance of nonspecific bands of different molecular weight in lanes may be caused by the lack of “hot start” or an inappropriate temperature regime in the thermocycler. In this case, the results of analysis are invalid.
- The appearance of the specific 520-bp band in lanes corresponding to negative controls (NCA, C–) suggests contamination of reagents or samples. In such cases, the results of analysis are considered to be invalid. Analysis of all samples must be repeated and

measures to detect and eliminate the source of contamination must be taken.

11. TRANSPORTATION

AmpliSens® *Helicobacter pylori*-EPh PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of **AmpliSens® *Helicobacter pylori*-EPh** PCR kit are to be stored at 2–8 ºC when not in use. All components of the PCR kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *Helicobacter pylori*-EPh** PCR kit is no less than $1x10^3$ 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 reagent kits DNA-sorb-B and EPh (manufactured by Federal Budget Institution of Science “Central Research Institute for Epidemiology”) are used.

13.2. Specificity

The analytical specificity of **AmpliSens® *Helicobacter pylori*-EPh** PCR kit is ensured by selection of specific primers and stringent reaction conditions. The clinical specificity was confirmed in laboratory clinical trials.

14. 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 Budget Institution of Science “Central Research Institute for Epidemiology”, Moscow, 2008.

15. QUALITY CONTROL.

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

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Upper limit of temperature	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+*Helicobacter pylori***	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
		PCE	Positive Control of Extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
11.01.11	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added.
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
	Content	The description of Negative Control was changed from «straw-colored clear liquid» to « colorless clear liquid»
		The volume of Negative Control was changed from 1.6 ml to 1.2 ml
	Through the text	Abbreviation for Positive Control DNA *Helicobacter pylori* was changed to C+$_{Helicobacter\ pylori}$
21.06.11 VV	Cover page, text	The name of Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”