



For *in Vitro* Diagnostic Use

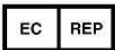
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AmpliSens® *Helicobacter pylori*-FRT

PCR kit

Instruction Manual



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1. INTENDED USE

AmpliSens® *Helicobacter pylori*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Helicobacter pylori* DNA in clinical material (biopsy material of gastric mucosa) by using real-time hybridization-fluorescence detection.



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 the pathogen genome specific region using specific *Helicobacter pylori* 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. **AmpliSens® *Helicobacter pylori*-FRT** 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. **AmpliSens® *Helicobacter pylori*-FRT** 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 a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Helicobacter pylori*-FRT PCR kit is produced in 1 form:

AmpliSens® *Helicobacter pylori*-FRT PCR kit variant FRT-50 F (for use with RG, iQ) **REF** R-B9(RG,iQ)-CE.

AmpliSens® *Helicobacter pylori*-FRT PCR kit variant FRT-50 F includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FL <i>Helicobacter pylori</i> / STI	colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube

Positive Control DNA <i>Helicobacter pylori</i> (C+^{Helicobacter pylori})	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 during the DNA extraction procedure directly to the sample/lysis mixture (DNA-sorb-B, **REF** K1-2-50-CE or RIBO-prep, **REF** K2-9-Et-50-CE).

AmpliSens® *Helicobacter pylori*-FRT 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 rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany), iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or equivalent).
- Disposable polypropylene microtubes for PCR (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 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 another 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 and then 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 is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.



The clinical material must be taken according to state and local authorities' requirements.

AmpliSens® *Helicobacter pylori*-FRT PCR kit is intended for analysis of DNA extracted by using DNA extraction kits from biopsy material of gastric mucosa.

7. PROTOCOL

7.1. DNA extraction

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

- DNA-sorb-B, **REF** K1-2-50-CE.
- RIBO-prep, **REF** K2-9-Et-50-CE.



Extract DNA according to the manufacturer's instructions.

7.2. Preparing PCR

7.2.1. Preparing tubes for PCR

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



Reaction mixture components should be mixed just before analysis with calculating for the required reaction number (test and control samples) according to Appendix 1. Note that even for analysis of one test or control DNA sample it is necessary to run all controls of the PCR amplification stage: positive controls (C+ and CS+) and negative control of amplification (NCA). It is recommended to mix the reagents for an even reaction number to ensure more exact dosage.

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.

2. Take the required number of tubes for amplification for the clinical and control samples. The type of tubes depends on the PCR instrument used for analysis.
3. To prepare the reaction mixture, mix **PCR-mix-1-FL *Helicobacter pylori* / STI**, **PCR-mix-2-FRT** and **Polymerase (TaqF)** in a new sterile tube (see Appendix 1). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.
4. Transfer **15 µl** of the prepared reaction mixture to each PCR tube.
5. Add **10 µl** of **DNA samples** obtained from the clinical samples. Dispose of the unused reaction mixture.



Avoid transferring sorbent beads together with the DNA sample in case of extraction with "DNA-sorb-B" reagents kit.

6. Carry out the control amplification reactions:

C+*Helicobacter pylori* -Add **10 µl** of **Positive Control DNA *Helicobacter pylori*** to the tube labeled C+*Helicobacter pylori* (Positive Control of Amplification).
 CS+ -Add **10 µl** of **Positive Control STI-88** to the tube labeled CS+ (Positive Control of Amplification of IC).
 NCA -Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

7.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

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

Table 1

AmpliSens-1 amplification program

Step	Rotor-type instruments ¹			Plate-type instruments ²		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
Hold	95	15 min	1	95	15 min	1
Cycling	95	5 s	45	95	10 s	45
	60	25 s		60	25 s	
		<i>fluorescent signal detection</i>			<i>fluorescent signal detection</i>	
	72	10 s		72	10 s	

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores on the 2nd step (60°C) of stage Cycling

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin*.

¹ For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.

² For example, iQ5, Mx3000P, Mx3000 or equivalent.

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. DATA ANALYSIS

Internal Control DNA is detected in the FAM/Green fluorescence channel,
Helicobacter pylori DNA is detected in the JOE/Yellow/HEX fluorescence channel.
 See **Guidelines** for data analysis settings for the instrument.

Interpretation of results

The results are interpreted by the software of used instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line.

The principle of interpretation is given in Table 2.

Table 2

Interpretation of amplification's results

Ct value in channel		Interpretation
FAM/Green	JOE/Yellow/HEX	
Ct value is defined	Pos (< boundary value*)	<i>Helicobacter pylori</i> DNA is detected
Pos (< boundary value*)	Neg (> boundary value*) or undefined	<i>Helicobacter pylori</i> DNA is not detected
Neg (> boundary value*) or undefined	Neg (> boundary value*) or undefined	Invalid result

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 (Table 3).

Table 3

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM/Green channel	JOE/Yellow/HEX channel	
C-	DNA extraction	Pos (≤ boundary value*)	Neg (> boundary value*) or undefined	OK
NCA	Amplification	Neg (> boundary value*) or undefined	Neg (> boundary value*) or undefined	OK
C+^{Helicobacter pylori}	Amplification	Pos (≤ boundary value*)	Pos (≤ boundary value*)	OK
CS+	Amplification	Pos (≤ boundary value*)	Neg (> boundary value*) or undefined	OK

*For boundary values, see the *Important Product Information Bulletin*

9. TROUBLESHOOTING

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

- If the Ct value is absent in both JOE/Yellow/HEX and FAM/Green channels or the Ct value in the JOE/Yellow /HEX channel is higher than the specified boundary value, PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (if iCycler iQ or iQ5 instruments are used).
- If the Ct value is present for C- in the FAM/Green channel and/or for NCA in the FAM/Green, JOE/Yellow/HEX channels in the results grid, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
- If no signal is detected for the positive controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components has not complied with the manufacturer's instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

10. STABILITY AND STORAGE

All components of the **AmpliSens[®] *Helicobacter pylori*-FRT** PCR kit (except for PCR-mix-1-FL *Helicobacter pylori* / STI, Polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens[®] *Helicobacter pylori*-FRT** PCR kit are stable until the expiration date on the label.



PCR-mix-1-FL *Helicobacter pylori* / STI, Polymerase (TaqF) and PCR-mix-2-FRT should be stored at ≤ -16 °C.



PCR-mix-1-FL *Helicobacter pylori* / STI should be kept away from light.

11. SPECIFICATIONS

11.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Helicobacter pylori*-FRT** PCR kit is the following:

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml ³
Biopsy material of gastric mucosa ⁴	DNA-sorb-B	1x10 ³
	RIBO-prep	1x10 ³

11.2. Specificity

The analytical specificity of **AmpliSens® *Helicobacter pylori*-FRT** 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. Nonspecific reactions were absent while testing human DNA samples and DNA panel of the following microorganisms: *Campylobacter jejuni ssp. jejuni* 43435, *C.fetus ssp. fetus* 25936, 20 strains of *C.jejuni*, 20 strains of *C.coli*, 5 strains of *C.lari*, 5 strains of *C.hyointestinalis* and 9 strains of *C.fetus*; *Salmonella enteritidis* S-6, *S.choleraesuis* 370, *S.typhimurium* 371, *S.dublin* 373, *S.typhi* C1, *S.abortusovis* 372 and *S.gallinarum-pullorum*; *Shigella flexneri* 851b; *Clebsiella* K 65 SW4; *Listeria monocitogenes* USKhCh 19 and *L. monocitogenes* USKhCh 52; *Proteus vulgaris* 115/98; *Pseudomonas aeruginosa* DN c1; *Staphilococcus aureus* 653 and *S.aureus* 29112; *Morganella morganii* 619 c 01; and *Enterobacter faecalis* 356. The clinical specificity of **AmpliSens® *Helicobacter pylori*-FRT** PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES

- Handbook "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.
- Guidelines "Real-Time PCR Detection of *Helicobacter pylori* DNA", 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.







³ Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

⁴ Pretreatment is not required.

13. QUALITY CONTROL

In compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Helicobacter pylori*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS

	Manufacturer		Temperature limitation
	Use by	LOT	Batch code
IVD	For <i>in Vitro</i> Diagnostic Use	VER	Version
REF	Catalogue number		Caution, consult accompanying documents
	Contains sufficient for <n> tests	CS+	Positive Control of Amplification of IC
	Consult instructions for use	NCA	Negative Control of Amplification
C+<i>Helicobacter pylori</i>	Positive control of amplification	C-	Negative control of extraction
CRIE	Central Research Institute of Epidemiology (Moscow, Russia)	IC	Internal Control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
15.09.10	Text	"Mucous coat of stomach" was changed to "gastric mucosa"
	Specificity	The names of microorganisms given in this section were abbreviated.
	References and Quality control	Central Research Institute of Epidemiology was quoted
24.10.10	Text	Positive Control DNA <i>Helicobacter pylori</i> (C+) was changed to Positive Control DNA <i>Helicobacter pylori</i> (C+ <i>Helicobacter pylori</i>)