AmpliSens® *Helicobacter pylori*-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Use-by Date
IVD	*In vitro* 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+*Helicobacter pylori*	Positive control of amplification
EC REP	Authorized representative in the European Community	IC	Internal control
	Caution	CS+	Positive Control of Amplification of IC

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 the clinical material (biopsy material of gastric mucosa) using real-time hybridization-fluorescence detection of amplified products.

NOTE: 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 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.

AmpliSens® *Helicobacter pylori*-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (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 the separation of nucleotides and Taq-polymerase by a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

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

Table 1

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

3. CONTENT

AmpliSens® *Helicobacter pylori*-FRT PCR kit is produced in 1 form:
variant FRT-50 F REF R-B9(RG,iQ)-CE.

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL *Helicobacter pylori* / STI	clear liquid from colorless to light lilac colour	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 *Helicobacter pylori* (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-FL (IC)** 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).

Variant FRT is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or equivalent).
- Disposable polypropylene PCR tubes:
  a) 0.2-ml tube (flat cap, nonstriped) for 36-well rotor if a rotor-type instrument is used.
  b) 0.2-ml tube (domed cap) if plate-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature 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

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

NOTE: 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 with DNA extraction kits from the clinical material (biopsy material of gastric mucosa).

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. 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.

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

In the extraction procedure it is necessary to carry out the control reaction as follows:

C– – Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

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

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

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

NOTE: Reaction mixture components should be mixed just before analysis with calculating for the required reaction number (including test and control samples) according to Table 2. Note that even for analysis of one test or control DNA sample it is necessary to carry out all controls of the 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 of 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 Table 2). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 2

Scheme of reaction mixture preparation

	Reagent volume for specified number of reactions		
Reagent volume per one reaction, µl	**10.00**	**5.00**	**0.50**
Number of reactions[1]	**PCR-mix-1-FL, µl**	**PCR-mix-2-FRT, µl**	**Polymerase (TaqF), µl**
8	**80**	**40**	**4.0**
10	**100**	**50**	**5.0**
12	**120**	**60**	**6.0**
14	**140**	**70**	**7.0**
16	**160**	**80**	**8.0**
18	**180**	**90**	**9.0**
20	**200**	**100**	**10.0**
22	**220**	**110**	**11.0**
24	**240**	**120**	**12.0**
26	**260**	**130**	**13.0**
28	**280**	**140**	**14.0**
30	**300**	**150**	**15.0**
32	**320**	**160**	**16.0**

4 Transfer **15 µl** of the prepared reaction mixture to each PCR tube.
5 Add **10 µl** of **DNA samples** obtained at the DNA extraction stage. Dispose of the unused reaction mixture.

NOTE: 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* (C+*Helicobacter pylori*)** to the tube labeled C+*Helicobacter pylori* (Positive Control of Amplification).

CS – Add **10 µl** of **Positive Control STI-88 (CS+)** 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).

C– – Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

8.2.2. Amplification

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

Table 3

AmpliSens-1 amplification program

	Rotor-type instruments[2]			Plate-type instruments[3]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	10 s	45	**95**	10 s	45
	60	25 s fluorescent acquiring		**60**	25 s fluorescent acquiring	
	72	10 s		**72**	10 s	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).
2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin* and Guidelines [2].
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.

[1] Number of clinical samples, control of DNA extraction (N), controls of amplification with one extra sample, (N+3+1).
[2] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[3] For example, iCycler iQ5, Mx3000P, Mx3000 or equivalent.

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:
– The signal of the IC DNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the *Helicobacter pylori* DNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the 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.
The principle of interpretation is given in Table 4

Table 4

Results interpretation

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

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 5).

Table 5

Results for controls

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

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:
1. If the *Ct* value determined for the Positive Control of amplification (C+) in the channel for the JOE fluorophore is greater than the boundary *Ct* value or absent, the amplification should be repeated for all samples in which *Helicobacter pylori* DNA was not detected.
2. If the *Ct* value determined for the Negative Control of extraction (C–) in the channel for the JOE fluorophore and/or the Negative Control of amplification (NCA) in the channel for the FAM and JOE fluorophores is less than the boundary *Ct* value, PCR should be repeated (starting from DNA extraction stage) for all samples in which *Helicobacter pylori* DNA was detected.

If you have any further questions or if encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Helicobacter pylori*-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FL *Helicobacter pylori* / STI, polymerase (TaqF) and PCR-mix-2-FRT). All components of the **AmpliSens® *Helicobacter pylori*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

NOTE: PCR-mix-1-FL *Helicobacter pylori* / STI, polymerase (TaqF), and PCR-mix-2-FRT are to be stored at the temperature from minus 24 to minus 16 ºC.

NOTE: PCR-mix-1-FL *Helicobacter pylori* / STI is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

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

13.2. Specificity

The analytical specificity of **AmpliSens® *Helicobacter pylori*-FRT** PCR kit is ensured by 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.
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.

[4] Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.
2. Guidelines to the **AmpliSens® *Helicobacter pylori*-FRT** PCR kit for qualitative detection of *Helicobacter pylori* DNA in clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for 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.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
19.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
24.10.14 ME	8.2.2. Amplification	In the amplification program for rotor-type instruments time was changed from 5 s to 10 s on the 1st step (95 °C) of stage Cycling
29.04.15 ME	Through the text	Corrections according to the template. Grammar corrections
	8.1. DNA Extraction	Information about controls of extraction was added
	8.2.1 Preparing tubes for PCR	Appendix 1 was integrated into the text of the instruction manual as Table 1
	9. Data analysis	The sections were rewritten
	10. Troubleshooting	
21.06.18 TA	3. Content	The color of the reagent was specified
07.05.20 KK	Through the text	The text formatting was changed
	2. Principle of PCR detection	The table with targets was added
	Footer	The phrase “Not for use in the Russian Federation” was added
18.03.21 EM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

EC REP

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