Instructions for Use (RUO)

In-vitro-

Diagnostic

IVD

The Helicobacter *direct* PCR is a real-time qPCR test for simultaneous *in vitro* detection of bacterial DNA of the pathogen ***Helicobacter pylori*** and its resistances against **levofloxacin** and **clarithromycin.**

Ref.No.:
FBC116-Ax

FBC116-Bx

The Helicobacter *direct* PCR was validated with the Roche LightCycler® 480 II and with the BioRad CFX Opus 96™. In general, Lab *direct* PCR tests are compatible with other qPCR cyclers (e.g. AJ qTOWER or LightCycler® 480 I).

Content

1. Name of the Device

Helicobacter *direct* PCR

2. Intended Purpose

The Helicobacter *direct* PCR is an assay for in vitro examination of bacterial DNA in biopsy, stool or culture samples to provide information to aid to diagnose patients under suspicion of infection with *Helicobacter pylori*. It furthermore allows for the detection of levofloxacin and clarithromycin resistances. Levofloxacin resistant *H. pylori* strains are differentiated via the mutations C261A/G, G271A/T and A272G in the *gyrA* gene. Clarithromycin resistance is detected by A2142G/C and A2143G mutations in the 23S rDNA.

The IVD medical device detects the DNA of the pathogen by qualitative measurements based on qPCR and is intended for use in medical laboratories or health institutions by laboratory personnel specifically trained in qPCR and in vitro diagnostic techniques. It has to be used in combination with conventional nucleic acid extraction systems for DNA extraction and qPCR cyclers for detection and analysis.

3. Pathogen Information

Helicobacter pylori (*H. pylori*) is a spiral-shaped, gram-negative bacterium that colonizes the human stomach. It is one of the most common chronic infections worldwide and is strongly associated with gastritis, peptic ulcer disease, and, in some cases, gastric cancer. *H. pylori* is typically acquired in childhood and can persist for decades if untreated. The bacterium survives the acidic environment of the stomach by producing urease, an enzyme that neutralizes gastric acid and allows it to inhabit the protective mucus layer (Hooi et al., 2017; Malfertheiner et al., 2017).

In recent years, antibiotic resistance has become a major challenge in the management of *H. pylori* infections. Standard treatment often combines a proton pump inhibitor with two or more antibiotics, but the success of these regimens has declined in many regions. Resistance to clarithromycin is particularly common and significantly reduces eradication rates. Resistance to metronidazole and levofloxacin is also increasing, complicating therapy choices. These trends vary by geographic location, making local resistance patterns an important factor in treatment planning (Megraud et al., 2013; Malfertheiner et al., 2017).

Clarithromycin resistance in *H. pylori* is primarily caused by point mutations in the 23S rRNA gene, most commonly A2142G, A2142C, and A2143G, which reduce macrolide binding to the bacterial ribosome (De Francesco et al., 2011). These mutations significantly impair the effectiveness of clarithromycin-containing regimens (Cambau et al., 2017). Levofloxacin resistance is mainly due to mutations in the quinolone resistance–determining region (QRDR) of the *gyrA* gene, particularly at positions N87 and D91, which alter DNA gyrase and prevent fluoroquinolone binding (Wu et al., 2012). The increasing prevalence of these mutations underscores the importance of molecular testing and tailored therapy in managing *H. pylori* infections.

4. Testing Principle

The Helicobacter *direct* PCR is a multiplex qPCR test for the detection of *Helicobacter pylori* and its resistances against levofloxacin and clarithromycin.

A separate Internal Control (IC) is added to each sample prior to DNA extraction and serves as control for nucleic acid isolation from the biological specimen as well as for amplification.

The nucleic acid eluate is added to the ready-to-use reaction solution that contains all reagents necessary for qPCR. The analysis can be performed on various qPCR cyclers.

The Helicobacter *direct* PCR contains primer and probes specific for the targets listed in Table 1 as well as the Internal Control. The probes are each labelled with fluorescent reporter dyes and a second dye that serves as a quencher and suppresses the fluorescence signals of intact probes.

Table 1: Analytes and target genes of Helicobacter direct PCR

ANALYTE	GENE	REGION (NUCLEOTIDES)
Helicobacter pylori	16S rDNA	896-966
Levofloxacin resistance	C261A/G, G271A/T and A272G in the *gyrA* gene	219-325
Clarithromycin resistance	A2142G/C and A2143G mutations in the 23S rDNA	2102-2220

The analysis is performed by determining Ct (cycle threshold) values. The Ct value describes the cycle in which the signal rises above a certain threshold for the first time. The more target copies are present in the sample, the lower the Ct value.

5. Package Content

Each kit contains the following vials which are sufficient for 96 reactions (see Table 2).

Table 2: Package content of Helicobacter direct PCR

MATERIAL	LID COLOUR	#VIALS; VOLUME	#RXNS.	COMMENT	
Solution A	green	1x; 1050 µL	96	Reaction mix	Polymerase (approx. 0.2 U/µl), primers and probes (approx. 50-150 nM)
Internal Control	blue	1x; 400 µL	96	Artificial nucleic acid target	Mix of RNA (approx. 10^7 copies/µl) and plasmid DNA (approx. 1 pg/µl)
Positive Control	red	1x; 50 µL	4	Nucleic acids of target analytes	Genomic DNA of *Helicobacter pylori* (approx. 580 copies/µl) and plasmid DNA with target region of *gyrA* (C261G mutation) and 23S rDNA (A2142C mutation)(approx. $3x10^3$ copies/µl)

6. Configurations

The Helicobacter *direct* PCR kit is available in the following variants:

REFERENCE NUMBER	CONFIGURATION	TARGETS
FBC116-Ax	suitable for use on a qPCR instrument with at least 5 detection channels (Cyan500/FAM/HEX/Red610/Cy5)	*H.pylori;* levofloxacin resistant *H.pylori;* clarithromycin resistant *H.pylori.*
FBC116-Bx	suitable for use on a qPCR instrument with at least 5 detection channels (FAM/HEX/Red610/Cy5/Cy5.5)	*H.pylori;* levofloxacin resistant *H.pylori;*

REFERENCE NUMBER	CONFIGURATION	TARGETS
		clarithromycin resistant *H.pylori.*

Table 3: Configurations of res4plex direct RT-PCR

7. Additional Equipment and Reagents (not provided)

- qPCR cycler (with at least 4 detection channels)
- Disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus lids/adhesive optical film
- Pipettes
- sterile filter-tips for PCR testing (DNA/RNA-free)
- Table centrifuge
- DNA extraction kit (MagNA Pure 96 DNA and Viral NA Small Volume Kit by Roche Life Science or similar device)
- Negative Control (no-template control, molecular grade water, or any other negative control according to the laboratory´s standard procedure)

8. Transport, Storage and Stability

The Helicobacter *direct* PCR is shipped on dry ice. All components must be stored at -25 °C to -18 °C in the dark immediately after receipt. After thawing, reagents should be kept at +2 °C to +8 °C before starting the test and used within 8 hours. Exposure to light should be avoided. You may thaw and refreeze the kit components up to 4 times. The package bears an expiry date, after which no quality guarantee can be given.

9. Warnings, Safety Precautions and Additional Information Access

The Helicobacter *direct* PCR is intended for *in vitro* diagnostic use only. The test should only be performed by personnel trained in molecular diagnostic techniques. If the user makes changes to the product or the application instructions, results may not correlate with the intended purpose.

All serious incidents relating to the kit must be notified to the manufacturer and the national competent authority of the EU Member State where the laboratory and/or patient is located.

- Before performing the test, read the entire instructions for use and follow them carefully. Deviations from the given test protocols can lead to invalid results.
- All patient samples must be treated as potentially infectious material.
- Discard sample and assay waste that was in contact with patient material according to your local, regional, or national safety regulations.
- Do not use the test beyond the expiration date.
- Do not use the test with opened or damaged packaging.
- Protect reagents from heat, moisture, and light.
- Do not replace or mix the reagents with reagents from other batches or other chemicals.
- Avoid contamination of the test by microorganisms and nucleases (DNases).
- Any carry-over of samples during handling and processing of the test may result in false positive test results.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner.
- Always wear disposable gloves in each area and change them before entering a different area.

- The test kits are intended for single use and must not be reused after performing qPCR reaction.
- If contamination of the qPCR cycler is suspected, cleaning and maintenance must be carried out according to the system´s manual.
- Safety Data Sheets (SDS) are available from FRIZ Biochem upon request. Please contact: order@frizbiochem.de.
- Pending EUDAMED entry, the Summary of Safety and Performance (SSP) can be obtained from FRIZ Biochem upon request. Please contact: order@frizbiochem.de.

10. Specimen Collection, Handling, Transport, and Storage

Proper sample collection, storage and transport are critical for PCR analyses in general. Inadequate sample collection, improper sample handling and/or transport can lead to false negative results. Therefore, ensure that:

- The instructions of the medical laboratory and/or the manufacturer of the sample collection system for sample collection, transport and storage are followed.
- The sample collector is instructed to collect the sample in accordance with applicable national laws, regulatory requirements and the instructions for use of the sampling tool.

11. Important Points before Starting

This test is intended for use with samples derived from biopsy, stool or culture. Other specimen types may cause invalid results.

In every qPCR run one Positive Control and one Negative Control should be included. The Positive Control consists of nucleic acids of the target gene regions. The Negative Control (e.g. molecular grade water) must be provided by the user. A failed Positive or Negative Control will invalidate the qPCR run and the results must not be reported.

Appropriate nucleic acid extraction methods must be conducted prior to using this assay. DNA extraction reagents are not part of the Helicobacter *direct* PCR kit. Performance evaluation studies have been conducted using the MagNA Pure 96 DNA and Viral NA Small Volume Kit on a MagNA Pure 96 instrument (Roche Life Science) for DNA isolation.

12. Test Procedure

Thaw all reagents completely and keep them cool (+2 °C to +8 °C) before starting the test, use within 8 hours, avoid exposure to light.

Table 4: Test procedure of Helicobacter direct PCR

TEST PROCEDURE	
Sample preparation	
1	Thaw all reagents completely.
2	Add Internal Control to the nucleic acid extraction in accordance with the laboratory´s standard procedure (e.g., add 4µL/sample to lysis buffer).
3	Perform nucleic acid extraction according to your laboratory's standard procedure.
qPCR	
4	Pipette 10 µL/well of Solution A into the PCR microtiter plate/reaction tubes.
5	Add 10 µL/well of eluate from nucleic acid extraction; add 10 µL Positive Control per run; add 10 µL Negative Control per run.
6	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
7	Briefly centrifuge the microtiter plate or reaction tubes.
8	Place the filled plate/reaction tubes in the qPCR cycler.
9	Start program.

13. Instrument Settings

The following thermal profile is to be used (Table 5). Channel settings depend on the qPCR cycler used and should be checked before starting the analysis (

Table 6). Make sure to activate the detection mode of your qPCR cycler before starting the analysis.

Table 5: Instrument settings of Helicobacter direct PCR

Steps	Temperature [°C]	Time	Acquisition	#Cycles
Initial step (optional)*	55	5 min	-	1
Denaturation	95	3 min	-	1
Amplification	95	5 sec	-	45
	60	15 sec	on	
	72	15 sec	-	

*If required, the amplification step can be adapted to a two-step cycling protocol by combining the two 60°C/15sec and 72°C/15sec steps into a single 60°C/30sec step.

Table 6: Channel settings of Helicobacter direct PCR

	Levofloxacin resistance	**Clarithromycin resistance**	**H.pylori wt**	**H.pylori wt**	**Internal Control**	
	gyrA	*23s rDNA*	*16s rDNA*	*23s rDNA**	artificial NA	
					FBC116-Ax	FBC116-Bx
Reporter dye	FAM	HEX	Red 610	Cy5	Cyan500	Cy5.5
Colour	green	yellow-green	orange	red	cyan	far-red
Emission	520	560	610	670	480	700
Quencher	Black Hole Quencher					

** As SNP detection is based on a single nucleotide difference, occasional low-level non-specific amplification in the HEX channel cannot be entirely excluded for wild-type samples. This extra channel is designed to resolve such ambiguous results and ensure reliable discrimination between wild-type and Clarithromycin-resistant H. pylori.*

The Helicobacter *direct* PCR was validated with the Roche LightCycler® 480 II and BioRad CFX Opus 96™. In general, FRIZ Lab *direct* PCR tests are compatible with many qPCR cyclers.

14. Results

Positive samples show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value (see Figure 1).

A positive signal is characterized by a sigmoidal curve exhibiting both a baseline phase and an exponential phase. Signals that do not exhibit this curve pattern, despite having a Ct value, are considered negative. The threshold shall be set within the exponential phase. It should be noted that in the case of low positive samples, the plateau phase might not be visible due to the cut off after 45 cycles. Thus, the presence of a plateau phase is not an essential requirement for defining a positive signal in such cases.

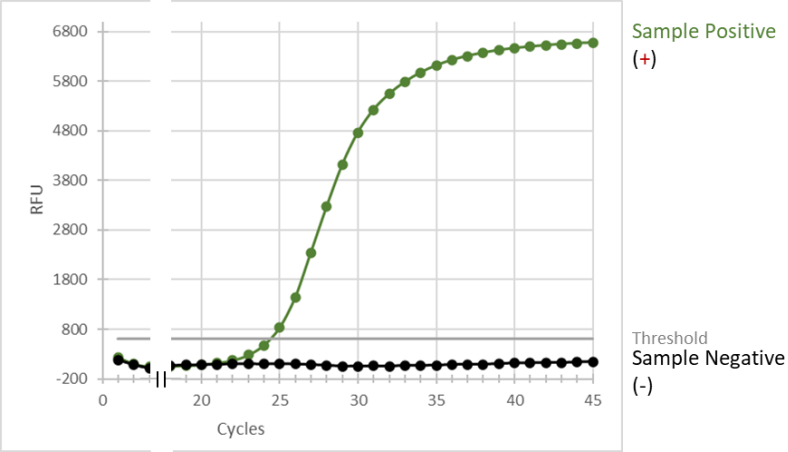

Figure 1: Exemplary amplification curves

- The Negative Control must not show amplification curves in all channels except for the IC channel.
- The Positive Control must show amplification curves in all channels. The Ct value of the Positive Control must be < 36. A Positive Control with a higher Ct value indicates procedural problems.
- The IC should show a positive amplification curve in both positive and negative samples.

For distribution of results within the channels see Table 7.

Table 7: Result interpretation of Helicobacter direct PCR

Levofloxacin resistance	**Clarithromycin resistance**	**H.pylori wt**	**H.pylori wt**		**Internal Control (IC)**			
gyrA	*23s rDNA*	*16s rDNA*	*23s rDNA*		*artificial NA*			
FAM	HEX	Red 610	Cy5		Cyan500 (FBC116-Ax)	Cy5.5 (FBC116-Bx)	**Result**	**Interpretation**
–	–	+	+		+		Valid	*H. pylori* wild-type detected.
+	–	+	–		+		Valid	*H. pylori* with levofloxacin resistance detected.
–	+	+	–		+		Valid	*H. pylori* with clarithromycin resistance detected.
+	+	+	–		+		Valid	*H. pylori* with levofloxacin and clarithromycin resistance detected.
+/–	+/–	–	+/–		+		Valid	No *H. pylori* detected.
+/–	+/–	+/–	+/–		–		Invalid	The test result cannot be evaluated.

15. Limitations of the Method

The results support the differential diagnosis of infections with *Helicobacter pylori* and its resistances against levofloxacin and clarithromycin. The bacterial DNA is generally detectable in biopsy samples of gastric mucosa and stool samples during both the acute and chronic phases of the infection.

Positive results indicate the presence of the respective pathogen, but do not exclude a co-infection with other pathogens.

A negative result does not exclude the presence of *H. pylori* or the resistances against levofloxacin or clarithromycin, as results depend on correct sampling, the absence of inhibitors and sufficient DNA to be detected. Invalid results may be obtained if the sample contains inhibitors that prevent lysis, extraction, amplification or detection of the target nucleic acids.

In the mutation detection channels (FAM, HEX), unspecific fluorescence signals may occur when detecting wildtype DNA. For reliable interpretation, results must always be evaluated in relation to the fluorescence signal of the 16S rDNA channel (Red 610) and the 23S rDNA wt channel (Cy5).

Test results should always be seen in the context of the clinical findings. Therapeutic consequences of the diagnostic results must be drawn in relation to the clinical findings.

The detection of analyte target does not mean that they are the causative agents of clinical symptoms.

Mutations or polymorphisms in primer and probe binding regions can interfere with the detection of new variants that may result in false negative results.

The detection of a target analyte does not exclude the possibility of a co-infection with another target analyte since competitive interference may occur (see Competitive Interference in the appendix).

16. Analytical Performance

16.1 Analytical Sensitivity

The limit of detection (LoD) was determined with serial dilutions of synthetic target-specific DNA. The analytes were tested in 20 replicates per concentration on a Bio-Rad CFX Opus 96™. The 95% confidence interval (95 CI) was determined using Logistic Regression (Logit) with the GraphPad Prism 9.3.1 software (see Table 7).

Table 8: Limit of Detection of Helicobacter direct PCR

ANALYTE	TARGET GENE	LIMIT OF DETECTION (95 CI)	UPPER LIMIT	LOWER LIMIT
H.pylori levofloxacin Resistance	*gyrA* C261A	22.95 copies/reaction	49.66	11.12
	gyrA C261G	15.78 copies/reaction	18.32	13.46
	gyrA G271A	13.76 copies/reaction	34.83	6.30
	gyrA G271T	15.32 copies/reaction	23.23	10.30
	gyrA A272G	21.06 copies/reaction	48.75	8.77
H.pylori clarithromycin Resistance	*23S rDNA* A2142C	13.82 copies/reaction	30.20	8.45
	23S rDNA A2142G	19.19 copies/reaction	35.08	11.02
	23S rDNA A2143G	26.71 copies/reaction	74.30	9.25
H.pylori	*16S rDNA*	5.87 copies/reaction	8.65	3.88

16.2 Other Analytical Performance Parameters

Other analytical performance data, such as analytical specificity including cross-reactivity, endogenous and exogenous interfering substances, inclusivity as well as precision (repeatability and reproducibility) were performed and demonstrated no impact on assay performance, with all test acceptance criteria met.

To note, according competitive interference results, this assay may not detect resistance mutations in specimens where a susceptible (wild-type) *H. pylori* population predominates over a resistant sub-population. Detection of minority resistant variants in mixed infections is not guaranteed. Clinical correlation is recommended in all cases, particularly where treatment failure occurs despite a susceptible result.

17. Diagnostic Performance

In total, 33 culture specimens were tested. Samples were extracted on a MagNA Pure 96 Instrument using the DNA and viral NA small volume kit (Roche). Eluates were tested with the Helicobacter *direct* PCR on a LightCycler® 480 II (Roche) and compared to a CE-certified test based on DNA-STRIP technology. In the event of discrepancy, results were resolved by sanger sequencing of the respective gene regions.

Diagnostic sensitivity and specificity are summarized in the following:

		***H. pylori* positive**		
		positive	**negative**	
Helicobacter *direct* PCR	**positive**	33	0	Sensitivity: 100.0% (89.4 – 100.0)
	negative	0	0	

		Levofloxacin-resistant *H. pylori*		
		positive	**negative**	
Helicobacter *direct* PCR	**positive**	3	0	Sensitivity = 100.0% (29.2 – 100.0)
	negative	0	30	Specificity = 100.0% (88.4 – 100.0)

		Clarithromycin-resistant *H. pylori*		
		positive	**negative**	
Helicobacter *direct* PCR	**positive**	12	0	Sensitivity = 100.0% (73.5 – 100.0)
	negative	0	21	Specificity = 100.0% (83.9 – 100.0)

The diagnostic sensitivity of the Helicobacter *direct* PCR is dependent on the DNA extraction method used to isolate DNA from biological specimens. It is the responsibility of the user to qualify the extraction methods used.

CE 0123 This product complies with the requirements of the Regulation (EU) *2017*/746 (*IVDR*) for in vitro diagnostic medical devices.

18. Symbols

Please note following symbol descriptions according to EN ISO 15223-1.

Graphic	Title	Description
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	Manufacturer	Indicate the medical device manufacturer.
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.
	Use-by date	Indicates the date after which the medical device is not to be used.
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.
	CE marking European Conformity	
	Catalogue number (Reference number)	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	Content sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.
	Do not reuse	Indicates a medical device that is intended for one single use only.
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Keep away from sunlight	Indicates a medical device that needs protection from light sources.
	Unique device identifier	Indicates a carrier that contains unique device identifier information.

Document Version:

FBC116_IFU_EN	
Version 1.0 Date: 2025-11-17	First version
Version 1.1 Date: 2026-02-02	• Chapter 5 Package Content: removal of "*One Positive Control/kit is included. If more Positive Control is needed, it can be purchased separately: Reference number FBC116-PC." • Chapter 8 Transport, Storage and Stability: "Reagents should be handled at +2 °C to +8 °C and used within 8 hours." was changed to "After thawing, reagents should be kept at +2 °C to +8 °C before starting the test and used within 8 hours." • Chapter 7 Configurations: removed due to one available configuration. • Chapter 12 Instrument Settings: Thermal profile adjusted to 63°C annealing temperature.
Version 2.1 Date: 2026-05-27	• Chapter 6 is newly added. • Chapter 13: Channel setting is updated • Chapter 14: Result interpretation is updated • Chapter 16: LoD data was updated

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19. References

Malfertheiner, P. et al. *Management of Helicobacter pylori infection—the Maastricht V/Florence Consensus Report.* **Gut** 66(1): 6–30, 2017.

Hooi, J.K.Y. et al. *Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-analysis.* **Gastroenterology** 153(2): 420–429, 2017.

Megraud, F. et al. *Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption.* **Gut** 62(1): 34–42, 2013.

De Francesco, V. et al. *Mechanisms of Helicobacter pylori antibiotic resistance: An updated appraisal.* **World Journal of Gastroenterology** 17(27): 3470–3481, 2011.

Cambau, E. et al. *Clarithromycin resistance in Helicobacter pylori: Molecular mechanisms and detection.* **Clinical Microbiology and Infection** 23(5): 355–362, 2017.

Wu, J.Y. et al. *Prevalence of gyrA mutations associated with fluoroquinolone resistance in Helicobacter pylori.* **Antimicrobial Agents and Chemotherapy** 56(1): 101–113, 2012.