Instructions for Use

RealLine Helicobacter pylori
Str-Format

KIT FOR THE QUALITATIVE DETECTION OF *HELICOBACTER PYLORI* DNA BY REAL-TIME PCR METHOD

In vitro Diagnostics

CE

RealLine Helicobacter pylori (Str Format)	**VBD3798**	**48 Tests**
valid from	**November 2019**	

BIORON Diagnostics GmbH
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Explanation of symbols used in labeling

IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Contains sufficient for <n> tests
	Use-by-date
	Temperature limit
	Consult instructions for use
	Keep away from sunlight
	Manufacturer

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ASSAY KIT FOR THE QUALITATIVE DETECTION OF *HELICOBACTER PYLORI* DNA BY REAL-TIME PCR METHOD
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1. INTENDED USE

Clinical Information:
Helicobacter pylori is a gram-negative, microaerophilic bacterium found in the stomach. The most people infected with *H.pylori* show no symptoms, but an acute infection may appear as an acute gastritis with abdominal pain (stomach ache) or nausea. Where an acute gastritis cause a chronic gastritis, the symptoms, are often those of non-ulcer dyspepsia: stomach pains, nausea, bloating, belching, and sometimes vomiting or black stool. The presence of *H.pylori* is a risk factor for gastric cancer.

RealLine Helicobacter pylori (Str-format) assay kit is designed to detect *Helicobacter pylori* DNA isolated from clinical specimens using extraction kits:
RealLine DNA-Extraction 2 (REF VBC8897)
RealLine Extraction 100 (REF VBC8896)

RealLine Helicobacter pylori (Str-format) kit is designed for the analysis of clinical materials: stomach tissue samples.

The assay is based on the real-time polymerase chain reaction (PCR) method with fluorescent detection of the amplified product.

The **Str-Format Kit** contains 48 tubes (0.2 ml) in strips with lyophilized Mastermix. 50 µl of extracted DNA have to be pipetted into the tube and the ready mastermix is diluted. The kit contains reagents required for 48 tests, including control samples and the positive control sample.

The kit is validated for use with: iQ™5 iCycler (Bio-Rad, USA). The kit is compatible with real-time PCR systems such as iQ™ iCycler, CFX™96 (Bio-Rad, USA), DT-96 (DNA-Technology, Russia) and RealLine Cycler (BIORON Diagnostics GmbH).

The use of:

- **Extraction Kits for nucleic acids from clinical specimen from other supplier**
- **other real-time PCR devices**
- **appropriate reaction volumes, other than 50 µl**

has to be validated in the lab by the user. The special notes regarding the internal control IC have to be strongly followed.

2. KIT CONTENTS

Positive Control sample (PC)	1 vial, 1 ml
Ready Master Mix (RMM), lyophilized	48 test-tubes (6 strips x 8 tubes)
Internal Control sample (IC), lyophilized	2 vials
The kit is additionally supplied with optical-transparent PCR-film	

Note: The RealLine Helicobacter Kit contains a special Internal Control Sample!

3. PRINCIPLE OF THE METHOD

The Real time PCR is based on the detection of the fluorescence, produced by a reporter molecule, which increases as the reaction proceeds. Reporter molecule is dual-labeled DNA-probe, which specifically binds to the target region of pathogen DNA. Fluorescent signal increases due to the fluorescent dye and quencher separating by Taq DNA-polymerase exonuclease activity during amplification. PCR process consists of repeated cycles: temperature denaturation of DNA, primer annealing and complementary chain synthesis.

Threshold cycle value – Ct – is the cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold, a fluorescent signal rises significantly above the background fluorescence. Ct depends on initial quantity of pathogen DNA template.
The use of **Internal Control (IC)** prevents generation of false negative results associated with possible loss of DNA template during specimen preparation. IC indicates if PCR inhibitors occur in the reaction mixture. IC template should be added in each single sample (including control samples) prior to DNA extraction procedure. The amplification and detection of IC does not influence the sensitivity or specificity of the target DNA PCR.

Note: IC is a component of the NA extraction kits of RealLine series. Internal Control is added to the sample during NA isolation step and is used throughout the whole process of NA extraction, amplification, detection.

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4. SPECIFICATIONS

4.1. Sensitivity – the detection of 100 *Helicobacter pylori* DNA copies in 5 samples prepared from the Standard Reference Sample (*Helicobacter pylori* DNA SRS) equals 100 %.

4.2. Specificity of *Helicobacter pylori* DNA detection using the Standard Reference Panel of negative samples equals 100 %.

4.3. Diagnostic sensitivity: clinical trials performed in two independent medical centers on 44 specimens (biopsy materials of gastric mucosa), containing *Helicobacter pylori* according to histology test, showed 100 % sensitivity (interval 93 % -100 % with a confidence level of 90 %).

4.4. Diagnostic specificity: clinical trials performed in two independent medical centers on 44 specimens (biopsy materials of gastric mucosa), not containing *Helicobacter pylori* according to histology test, showed 100 % sensitivity (interval 93 % -100 % with a confidence level of 90 %).

Analysis by the CE-marked reference kit showed full match of results.

5. PRODUCT USE LIMITATIONS

- This assay must not be used on the clinical specimen directly. Appropriate nucleic acids extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- When monitoring a patient the same extraction method must be used in all determinations. Otherwise, results may not be comparable.
- The kit is designed for use in patients with a clinical history and/or symptoms consistent with *Helicobacter pylori.* infections. The kit may be used for screening purposes.
- Diagnostic sensitivity of the kit may vary depending on the pathogen prevalence and characteristics of the enrolled cohort.
- Reliable results depend on adequate specimen sampling.
- Positive results indicate active or asymptomatic infection; clinical history and symptoms should be taken into account.
- Negative results indicate lack of detectable DNA but do not exclude the infection or disease.
- Potential mutations within the target regions of the *Helicobacter pylori* genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- The kit is not intended to replace culture and other methods (e.g., cervical exam) for diagnosis of infections.

6. WARNING AND PRECAUTIONS

- For in vitro use only.
- The kits must be used by skilled personnel only.
- When handling the kit, follow the national safety requirements for working with pathogens.
- To prevent contamination, the stages of DNA isolation and PCR test run must be spatially separated.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent vials.
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents.
- Every workplace must be provided with its own set of variable-volume pipettes, necessary auxiliary materials and equipment. It is prohibited to relocate them to other workplaces.
- The use of sterile disposable pipette tips is recommended.
- Never use the same tips for different samples.
- Do not pool reagents from different lots or from different vials of the same lot.
- Dispose unused reagents and waste in accordance with country, federal, state and local regulations.
- Do not use the kit after the expiration date at the side label of the box.

7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- real time PCR system, like described in p.1
- DNA-Extraction Kit, see p.1 Extractions Kits;
- safety laminar box;
- refrigerator;
- half-automatic variable-volume single-channel pipettes;
- disposable medical non-sterile powder-free gloves;
- disposable pipette tips with aerosol barrier;
- biohazard waste container;
- razor or scalpel.

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8. PREPARATION OF THE SPECIMEN

The assay is performed on extracted DNA samples obtained from the clinical material using one of the DNA extraction kits listed in p.1, according to the Instruction Manual to the kit.

*Each group of samples undergoing the procedure of DNA isolation must include a **Positive Control sample (PC)** from this kit and a **Negative Control sample (NC)** which is a component of the DNA extraction kit.*

We strongly recommend the implementation of the Internal Control IC, the Negative Control NC and Positive Control PC samples to the extraction procedure.
When using kits of another supplier for the extraction of nucleic acids as recommended in chapter 1: add **20 µl** of **IC (VBC8881)** to each tube.

- For the NC use **100 µl** of the Negative Control Sample
- For the PC use **70 µl** of Negative Control Sample and **30 µl** of Positive Control to the tube marked PC.

If samples of isolated DNA were stored frozen prior the assay, thaw them and keep at least 30 minutes at a temperature of (18 – 25) °C.

The isolated DNA can be stored at (2 – 8) °C for 24 hours.
After initial opening shelf life of Positive Control sample is 1 month at (2 – 8) °C or for 50 µl aliquots 3 month at (-18 … -60) °C

9. PROCEDURE

9.1. Preparation of the kit components

Prior to the test, take the kit out of the refrigerator and keep the **Ready Master Mix (RMM)** closed in the package at (18 – 25) °C for at least 30 min. Then open the package and cut off the necessary number of tubes with RMM (including the specimens and control samples) with the razor or scalpel. Cut the tubes together with the covering film.
Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close it with a clip.
After initial opening, store RMM at (2 – 8) °C for no more than 3 months.

In the process of *Helicobacter pylori* DNA extraction use IC sample provided with the kit.
Add 1000 µl of Recovery Solution for Control samples (RSC), to the tube with IC, mix gently, keep at (18 – 25) °C during 15 min, and mix thoroughly once again. RSC is the component of DNA extraction kit.

Store prepared IC at (2-8) °C for no more than 1 months.
Perform *Helicobacter pylori* DNA extraction in accordance with Instruction Manual to the kit.

9.2. Label the tubes with RMM for each specimen and control.
Attention! *Labels should be placed on the lateral side of the tubes.*

9.3. Add **50 µl** of corresponding isolated DNA solution to each tube using a separate pipette tip with filter. Tightly close the tubes with caps or seal with the PCR transparent film.

9.4. Place the tubes into the real-time PCR system.

9.5. Program real time PCR system as follows:

Step 1:	50°C	2min	
Step 2:	95°C	2min	
Step 3:	94°C	10 sec	50 cycles
	60°C*	20 sec	

* Measure the fluorescence at 60°

9.6. Select the amplification detection channels:

- Collect real-time PCR data through the **FAM** channel for detection of amplification of IC DNA.
- Collect real-time PCR data through the **ROX** channel for detection of amplification of *Helicobacter pylori* DNA.

9.7. Program the positions of test tubes with samples, positive and negative controls according to the instruction manual for the real time PCR system in use.

9.8. Run the program.

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10. DATA ANALYSIS AND INTERPRETATION

10.1 For **PC** the program should detect:

- increase of the IC DNA amplification signal (channel **FAM**) and determine the threshold cycle, IC **Ct**;
- increase of the *Helicobacter pylori* DNA amplification signal (channel **ROX**) and determine the **Ct** value;

10.2 For **NC** the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine the threshold cycle, IC **Ct**. No **ROX** fluorescent increase should appear (*no Helicobacter pylori DNA amplification*).

10.3 For each specimen the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine IC **Ct**.

10.4 Calculate (IC **Ct**)$_{av}$ as an average IC **Ct** of all analyzed samples (including PC and NC). IC **Ct** values that differ by more than 2 cycles from the (IC **Ct**)$_{av}$ should be ignored. Recalculate the (IC **Ct**)$_{av}$ for the remaining values after the screening.

10.5 The sample is considered **positive**, i.e. contains *Helicobacter pylori* DNA, when **Ct** value via **ROX** channel for this sample is **less than or equals to 40.**

10.6 The sample is considered **negative** (not containing *Helicobacter pylori* DNA), if **Ct** value via **ROX** channel for this sample is **above 40** or is not determined.

If IC **Ct** value for such sample differs from the (IC **Ct**)$_{av}$ value by more than 2 cycles, the result is regarded as equivocal. A repeated analysis of the sample, starting with the DNA isolation step is necessary.

10.7 If the Ct value for NC through the **ROX** channel is less than or equal to 40, it indicates the presence of contamination. In case of contamination all positive results of this individual PCR test run are considered as **equivocal**. Actions are required to identify and eliminate the source of contamination, and repeat the analysis of all samples of this run that were identified positive. Samples that showed negative results in this run should be considered as negative.

11. STORAGE AND TRANSPORTATION

- Store and transport the assay kit at (2 – 8) °C in the manufacturer's packing.
- Transportation at 25°C for up to 10 days is allowed.
- Do not freeze the kit!
- Do not pool reagents from different lots or from different vials of the same lot.
- Strictly follow the Instruction manual for reliable results.
- Do not use kits with damaged inner packages and get in contact with BIORON Diagnostics GmbH.
- **Storage and shelf life of solutions and components of the kit after initial opening:**
  Positive Control sample: 1 month at (2 – 8) °C C *or in 50 µl aliquots at minus (18 - 60) °C for up to 3 months*.
  Ready Master Mix (RMM): 3 months at (2 – 8) °C
  Diluted IC: 1 month after preparation at (2 – 8) °C

Technical Support: techsupport@bioron.de

ANNEX I: Settings for RealLine Cycler and DT96:

for these cyclers the measurement exposure must be adjusted. Choose the **Operation with the device** mode in the **Settings** menu, select the item **Measurement exposition:**

- **FAM** to **250**
- **HEX** and **ROX** to **1000**

Confirm that the current exposure value is saved by pressing **YES**

Attention! The specified exposure values are applicable only for RealLine kits and, if necessary, must be changed for other purposes.

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