Helicobacter Pylori PCR Kit

Multiplex real-time PCR assay for the direct, qualitative detection of *Helicobacter pylori* and its resistance to clarithromycin from human native tissue biopsy material.

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 86-HPYHU-100
Size: 100 determinations
Version: 2018-11-12 - ALPCO 1.0

INTENDED USE

The Helicobacter pylori PCR kit is a multiplex real-time PCR assay for the direct, qualitative detection of *Helicobacter pylori (H. pylori)*.[1] and its resistance to clarithromycin from human native tissue biopsy material. For Research Use Only. Not for use in diagnostic procedures.

PRINCIPLE OF THE TEST

The Helicobacter Pylori PCR kit is a multiplex real-time PCR assay for the direct, qualitative detection of *H. pylori* from human biopsy samples. After DNA-isolation, amplification of gene fragments (if present) specific for *H. pylori* (16S rRNA) and a potential resistance to clarithromycin (23S rRNA) occurs.

The amplified targets are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target, the probes hybridize to the amplicons. During the extension step, the Taq-polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescence signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the number of formed amplicons. The Helicobacter Pylori assay contains an Internal Control DNA (ICD) as an internal control of the sample preparation procedure and to determine possible PCR inhibition.

REAGENTS PROVIDED

Reagents provided in the kit are sufficient for 100 determinations.

Table 1. Reagents Provided

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 uL	yellow
2	Taq-Polymerase	1x	80 uL	red
D	Internal Control DNA	2x	1700 uL	orange
N	No Template Control	1x	450 uL	white
P	Positive Control	1x	200 uL	blue

STORAGE INSTRUCTIONS

- Protect all reagents from light and store at -20°C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before use (e.g., in a refrigerator at 2 - 8°C).
- Reagents can sustain up to 20 freeze/thaw cycles without influencing the assay performance. After the initial thaw, separate into aliquots and freeze immediately.
- During PCR preparation, all reagents should be stored cold (2 - 8°C).

MATERIALS REQUIRED BUT NOT PROVIDED

The Helicobacter Pylori multiplex real-time PCR assay is suitable for use with the following extraction platforms and PCR instruments. If using another PCR instrument, refer to the settings for the tested instruments as a starting point and modify as necessary.

Table 2. Extraction Platforms and PCR Instruments

Extraction platform	
ALPCO	DNA/RNA Extraction Kit
Promega	Maxwell® RSC
Roche	MagNA Pure 96
Real-time PCR instrument	
Roche	LightCycler® 480II
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q

Note: Only use 0.1 mL tubes on the Rotor-Gene Q from Qiagen.

- Extraction platform if extraction not performed manually
- Real-time PCR instrument
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortex mixer
- Pipettes (0.5 - 20 uL, 20 - 200 uL, 100 - 1000 uL)
- Filter tips
- Powder-free disposal gloves
- PCR Grade water (bioscience grade, nuclease-free)
- Color Compensation Kit IV (if running the LightCycler® 480II)

WARNINGS and PRECAUTIONS

- Good laboratory practices must be used and the instructions for carrying out the test must be strictly followed.
- Do not mix reagents from kits with different lot numbers.
- Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes.

- During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure.
- Do not smoke, eat or drink in areas where samples or reagents are being used.
- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.
- All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

PREPARATION OF SAMPLES

Sample preparation from biopsy material

For DNA isolation of human biopsy samples, use a commercially available DNA isolation kit (e.g., DNA/RNA Extraction Kit (ALPCO)) or DNA extraction system (e.g., Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions. We recommend incubating the biopsy sample overnight at 55°C using Proteinase K before extraction. From this sample, use the appropriate volume for extraction according to the manufacturer's instructions.

The Helicobacter Pylori PCR assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as both an extraction control for the sample preparation procedure and a PCR inhibition control.

If the Internal Control DNA is used only as a PCR inhibition control, 1 uL of the Internal Control DNA should be added to the Master Mix (see Table 4). If the Internal Control DNA is used as an extraction control for the sample preparation procedure and as a PCR inhibition control, 20 uL of the Internal Control DNA must be added during extraction procedure. The Internal Control DNA should always be added to the specimen-lysis buffer mixture and must not be added directly to the specimen. It is also recommended to add 1 uL of the Internal Control DNA to the negative control and positive control PCR Mix.

MASTER MIX PREPARATION

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and one negative control must be included in each assay run. It is recommended to add a 10% volume overage to compensate for imprecision in pipetting (see Tables 3 and 4). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Taq-Polymerase, the Positive Control, the *No Template Control* and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 - 8°C).

Table 3. Calculation and pipetting example for 10 reactions of the Master Mix (ICD as extraction and PCR inhibition control)

Kit code	Master Mix Components	Volume per Reaction	10 reactions (+10% overage)
1	Reaction Mix	19.3 uL	212.3 uL
2	Taq-Polymerase	0.7 uL	7.7 uL
	Total Volume	**20 uL**	**220 uL**

Table 4. Calculation and pipetting example for 10 reactions of the Master Mix (ICD only as PCR inhibition control)

Kit code	Master Mix Components	Volume per Reaction	10 reactions (+10% overage)
1	Reaction Mix	19.3 uL	212.3 uL
2	Taq-Polymerase	0.7 uL	7.7 uL
D	Internal Control DNA	1.0 uL	11 uL
	Total Volume	**21 uL**	**231 uL**

Mix the components of the Master Mix gently and briefly spin down.

PREPARATION OF THE PCR MIX

1. Pipette 20 uL of the Master Mix into all reaction wells (tubes or plate wells).

2. Add 5 uL of the *No Template Control* to the negative control wells.

Note: If the Internal Control DNA is being used as extraction control for the sample preparation procedure and as PCR inhibition control, add 1 uL of the Internal Control DNA to the negative control vial.

3. Add 5 uL of DNA-Extract to corresponding sample wells.

4. Add 5 uL of Positive Control to the positive control wells.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, add 1 uL of the Internal Control DNA to the positive control wells.

5. Cover tubes or plate. Spin down and place in the real-time PCR instrument. The RT-PCR reaction should be started according to the PCR instrument set-up (see Table 5, 6, 7, and 8).

PCR INSTRUMENT SET-UP

Table 5. DNA Real-time PCR Profile for LightCycler™ series and Rotor-Gene Q

Initial Denaturation	1 min, 95°C
Cycles	45 cycles
PCR Denaturation PCR Annealing / Extension	10 sec, 95°C 15 sec, 60°C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step

Table 6. DNA Real-time PCR Profile for Mx3005P, ABI7500, CFX96™

Initial Denaturation	1 min, 95°C
Cycles	45 cycles
PCR Denaturation PCR Annealing / Extension	15sec, 95°C 30sec, 60°C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step

The Universal real-time PCR profiles outlined below should only be used for DNA assays when combining DNA and RNA real-time PCR assays in one run.

Table 7. Universal Real-time PCR Profile for LightCycler™ series

Reverse Transcription	10 min, 58°C
Initial Denaturation	1 min, 95°C
Cycles	45 cycles
PCR Denaturation PCR Annealing / Extension	10 sec, 95°C 15 sec, 60°C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step

Table 8. Universal Real-time PCR Profile for Mx3005P, ABI7500, CFX96™ and Rotor-Gene Q

Reverse Transcription	10 min, 58°C
Initial Denaturation	1 min, 95°C
Cycles	45 cycles
PCR Denaturation PCR Annealing / Extension	15sec, 95°C 30sec, 60°C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step

DETECTION CHANNEL SET-UP

Table 9. Selection of Appropriate Detection Channels

Real-time PCR Instrument	Detection	Detection Channel	Note
Roche LightCycler® 480II	*H. pylori*	465/510	Color compensation kit IV is required.
	ICD	533/580	
	Clarithromycin resistance	618/660	
ABI 7500	*H. pylori*	FAM	Check that passive reference option ROX is none
	ICD	VIC	
	Clarithromycin resistance	Cy5	
Agilent Techn. Mx3005P	*H. pylori*	FAM	Check that reference dye is none
	ICD	HEX	
	Clarithromycin resistance	Cy5	
Qiagen Rotor-Gene Q	*H. pylori*	Green	The gain settings have to be set to 5, according to the default settings
	ICD	Yellow	
	Clarithromycin resistance	Red	
Bio-Rad CFX96™	*H. pylori*	FAM	-
	ICD	VIC	
	Clarithromycin resistance	Cy5	

QUALITY CONTROL

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer's instructions. Negative control and positive control have to show correct results (see Table 10, Fig. 1, Fig. 2) in order to determine a valid run.
The **Positive Control** has a concentration of 10^3 copies/uL. In each PCR run it is used in a total amount of 5 x 10^3 copies.

Table 10. For a valid run, the following conditions must be met:

Sample	Assay result	ICR Ct	Target Ct
Positive Control	Positive	NA *[1]	See Quality Assurance Certificate
Negative Control	Negative	Ct > 20	0

*[1] *No Ct value is required for the ICD to make a positive call for the Positive Control*

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid prepare all new reactions including the controls.

If the required criteria are not met, the following items should be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

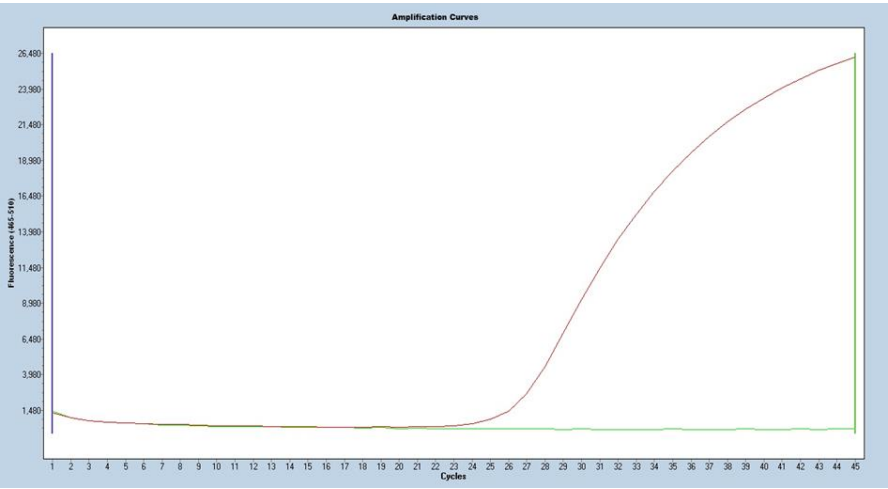

Figure 1: Correct run of the positive and negative controls (*Helicobacter pylori*) on the LightCycler® 480II

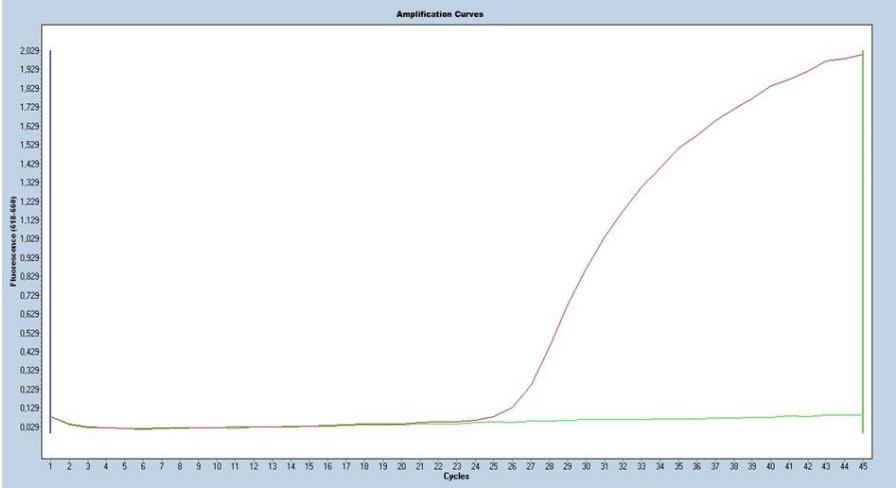

Figure 2: Correct run of the positive and negative controls (Clarithromycin resistance) on the LightCycler® 480II

RESULTS INTERPRETATION

The result interpretation is done according to Table 11.

Table 11: Sample interpretation

Helicobacter pylori	**Clarithromycin resistance**	**ICD**	**Result**
positive	negative	**positive / negative**	*H. Pylori* detected
positive	**positive***	**positive / negative**	*H. Pylori* and Clarithromycin resistance detected
negative	**positive***	**positive / negative**	*H. pylori* not detected
negative	negative	**positive**	Target genes not detected
negative	negative	negative	Invalid

***Note:** When using the LightCycler® 480II (Roche) or the CFX96™ (Biorad), the fluorescence signal of a true positive signal in the clarithromycin resistance channel (Cy5) has to be more than 20% of the fluorescence signal of the positive control or when using the Mx3005P (Agilent Technologies), the ABI 7500 (Applied Biosystems) or the Rotor-Gene Q (Qiagen), the fluorescence signal has to be more than 10% of the fluorescence signal of the positive control. For a clearer evaluation, we recommend setting the threshold on this value at 10% or 20% of the fluorescence signal of the positive control.

A sample is evaluated negative if the sample shows no amplification signal in the detection system, but the Internal Control DNA is positive. An inhibition of the PCR reaction or a failure in the extraction procedure can be excluded by the detection of the Internal Control DNA.

A sample is evaluated positive if the sample shows an amplification in the detection system, but the Internal Control DNA is negative. The detection of the internal amplification control is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the internalamplification of the control..

A sample is evaluated invalid if both the sample and Internal Control DNA show no amplification signal in the detection system. The sample contains a PCR inhibitor or a failure occurred in the extraction procedure. The extracted sample needs to be further diluted with PCR grade water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

LIMITATIONS OF THE METHOD

1. This assay is For Research Use Only. It is not for use in diagnostic procedures.
2. This assay is only validated for biopsy material.
3. Inappropriate specimen collection, transport, storage and processing or a viral load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect new variants resulting in a false negative result with the Helicobacter pylori assay.
6. As with all PCR based tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target genes (16S rRNA, 23S rRNA).
8. In individual cases, weak cross-reactivity can occur in the Clarithromycin channel in presence of organisms that also carry the Clarithromycin wildtype genome but which are not *Helicobacter pylori*.
9. In individual cases, cross-reactivity can occur in the *H. pylori* channel in presence of *Helicobacter felis* though *H. pylori* is not present in the sample (cross-reactivity).

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The Helicobacter Pylori PCR multiplex real-time assay has a detection limit of ≥ 10 DNA copies per reaction. The following figures 3 and 4 show a dilution series of H. pylori and the Clarithromycin resistance (each 10^5– 10^1 DNAcopies per uL) on the LightCycler® 480II.

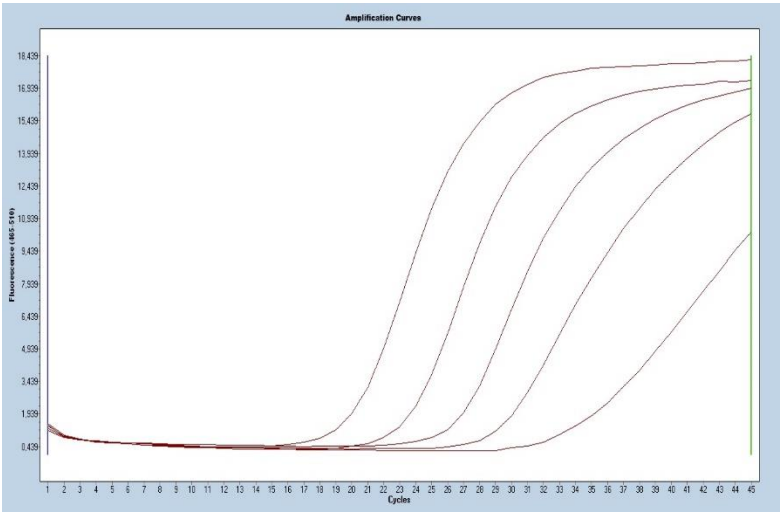

Fig.3: Dilution series *H. pylori* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

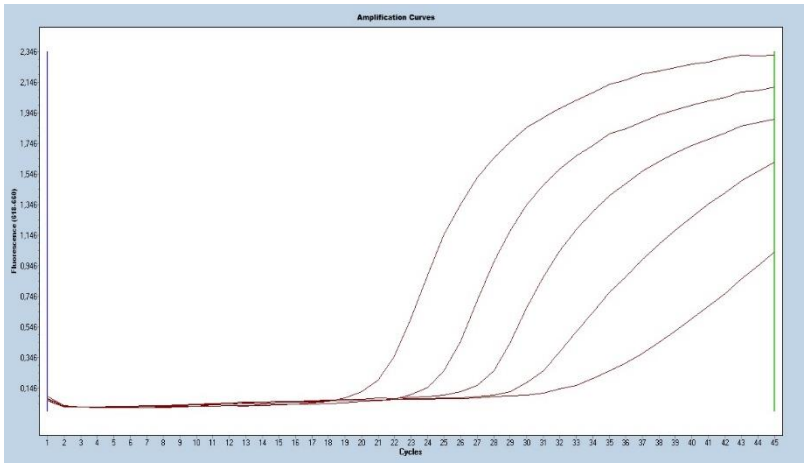

Fig.4: Dilution series Clarithromycin resistance (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

Analytical Specificity

The analytical specificity of the Helicobacter Pylori multiplex real time PCR assay is specific for *Helicobacter pylori* from human biopsy samples. No cross-reaction could be detected for the following species (see Table 12):

Table 12: Cross-reactivity Testing

Adenovirus 40, human, strain Dugan	-	*Campylobacter upsaliensis*	-	*Enterobacter cloacae*	-	*Pseudomonas aeruginosa*	-
Adenovirus 41, human, strain Tak	-	*Candida albicans*	-	*Enterococcus faecalis*	-	Rotavirus	-
Aeromonas hydrophila	-	*Citrobacter freundii*	-	*Giardia intestinalis* Portland 1	-	*Salmonella enteritidis*	-
Arcobacter butzleri	-	*Clostridium difficile*	-	*Giardia intestinalis* WB Clone C6	-	*Salmonella typhimurium*	-
Astrovirus	-	*Clostridium perfringens*	-	*Klebsiella oxytoca*	-	*Serratia liquefaciens*	-
Bacillus cereus	-	*Clostridium sordellii*	-	*Listeria monocytogenes*	-	*Shigella flexneri*	-
Bacteroides fragilis	-	*Cryptosporidium parvum*	-	Norovirus GGI	-	*Staphylococcus aureus*	-
Campylobacter coli	-	*E. coli (O157:H7)*	-	Norovirus GGII	-	*Vibrio parahaemolyticus*	-
Campylobacter jejuni	-	*E. coli (O26:H-)*	-	*Proteus vulgaris*	-	*Yersinia enterocolitica*	-
Campylobacter lari subsp. *lari*	-	*E. coli (O6)*	-		-		-

REFERENCES

1. Rimbara E. PCR Detection of Helicobacter pylori in clinical samples. Methods Mol. Biol. 2013, 943: 279-287

2. Glocker E, et al. Quinolone resistance in Helicobacter pylori isolates in Germany. Antimicrob. Agents Chemother. 2007, 51(1): 346-349

3. O'Connor A. Treatment of Helicobacter pylori infection in 2010. Helicobacter ISSN 1523-5378, Helicobacter 15 (Suppl. 1): 46-52