BactoReal® Kit *H. pylori* ClariRes

Instructions for Use

CE

IVD **For *in vitro* diagnostic use**

REF **DHUB1000**

Σ **50 reactions**

ingenetix GmbH
Haidingergasse 1
1030 Vienna, Austria
T +43(0)1 36 1980 198
F +43(0)1 36 1980 199
office@ingenetix.com
www.ingenetix.com

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Explanation of symbols

LOT	Batch code		Use by date
REF	Catalogue number		Manufacturer
Σ	Contains sufficient for <n> tests		Temperature limit (Store at)
CE	This product fulfils the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices	IVD	In vitro diagnostic medical device
	Consult instructions for use	UDI	Unique device identifier
	Keep away from sunlight	QTY	Contents
	Corrosion, GHS05		Exclamation mark, GHS07

1. Intended use

BactoReal® Kit *H. pylori* ClariRes is a non-automated IVD test, based on real-time polymerase chain reaction (PCR), for the qualitative detection of DNA (23S rRNA Gen) of *Helicobacter pylori* (*H. pylori*) in combination with the detection of a possible clarithromycin susceptibility. The test allows detection of a *H. pylori* infection with simultaneous detection of the wild-type (susceptible to clarithromycin) as well as of three most common point mutations (A2142C, A2142G, and A2143G) in the 23S rRNA gene of *H. pylori* responsible for resistance to treatment with clarithromycin.
Proper specimens are DNA extracts from human gastric tissue biopsies and fresh or frozen stool samples without pre-enrichment.
This test is suitable for patients of all ages with suspected infection with *H. pylori* and is intended as an aid in the diagnosis of infection with this pathogen in combination with patient history and additional clinical information.

The test is for professional use only and the use is limited to qualified personnel instructed in real-time PCR and *in vitro* diagnostic procedures.

2. Product description

BactoReal® Kit *H. pylori* ClariRes is a real-time PCR test and detects the 23S rRNA gene of *H. pylori*. This test amplifies a region in the 23S rRNA gene of *H. pylori* that contains the three most common point mutations (A2142C, A2142G, and A2143G) responsible for clarithromycin resistance in *H. pylori* by real-time PCR. Strains containing the wild-type sequence of the 23S rRNA gene are sensitive to treatment with clarithromycin. The wild-type sequence and the mutants are detected and distinguished by melting curve analysis in the FAM/SYBR® Green channel following amplification (see 11. interpretation of the PCR-data).

The internal DNA positive control (IC) is also detected in the channel for FAM/SYBR® and is used as DNA extraction and/or as real-time PCR inhibition control. The target for the IC (artificial target DNA) is extracted with the sample or is added to the PCR reaction.

BactoReal® Kit *H. pylori* ClariRes is compatible with the real-time PCR instruments LightCycler® 1.1/1.2/1.5 oder 2.0 Instrument (Roche), LightCycler® 480 (Roche), instruments of the Quantstudio™ series (Thermo Fisher Scientific), ABI® 7500 Real-Time PCR System (Thermo Fisher Scientific) and Magnetic Induction Cycler (MIC; Bio Molecular Systems). Other instruments which are capable of detecting FAM/SYBR® Green and performance of melt curve analysis should be validated for compatibility with the kit.

3. Pathogen information

Helicobacter pylori colonizes the human stomach and is associated with gastritis and gastroduodenal ulcer and gastric cancer. At present, several diagnostic tests for *H. pylori* detection are available. Invasive methods requiring gastric endoscopy include rapid urease testing, culture, histology and molecular diagnostics. Non-invasive approaches include faecal antigen detection, serologic testing, urea breath testing and molecular diagnostics. Infection with *H. pylori* can be effectively treated with proton pump inhibitors and various antibiotics. Clarithromycin is an integral part of first line therapies to treat *H. pylori* infection. Since clarithromycin is a widely used antimicrobial drug, the prevalence of clarithromycin resistant *H. pylori* strains is increasing continuously. Resistance to clarithromycin is mainly due to three major point mutations at two positions (A2142C, A2142G, and A2143G) within the peptidyltransferase region of the 23 S rRNA of *H. pylori*.

References:

Claudia Schabereiter-Gurtner, Alexander M. Hirschl, Brigitte Dragosics, Peter Hufnagl, Sonja Puz, Zsuzsanna Kovách, Manfred Rotter and Athanasios Makristathis. 2004. Novel real-time PCR assay for detection of *Helicobacter pylori* infection and simultaneous clarithromycin susceptibility testing in stool and biopsy specimens. J. Clin. Microbiol. 42:4512-8.

4. Principle of real-time PCR

Detection by real-time PCR involves amplification of specific regions of the pathogen genome. The PCR products are detected by fluorescent dyes coupled to oligonucleotide probes.
After PCR amplification, a melting curve analysis is performed. In case of a wild-type target sequence the probe dissociates at its melting temperature (Tm). In case of a mutation in the target sequence, it dissociates at lower temperature. This technology allows the sequence-specific detection of the 23S rRNA gene of *H. pylori* and the determination of the three major point mutations in the PCR-product.

5. Contents of the kit, stability and storage

Labelling	Content	Amount	Storage
H. pylori Assay Mix (green cap)	Primers and probe for detection of - *H. pylori* - IC (internal positive control)	1 x 50 µl	-25 °C to -15 °C
H. pylori IC Target (orange cap)	Internal positive control (IC)	1 x 50 µl	-25 °C to -15 °C
H. pylori Wild-type Positive Control (red cap)	Control-DNA *H. pylori* 23S rRNA wild-type (sensitive to clarithromycin)	1 x 300 µl	-25 °C to -15 °C
H. pylori A2142G Positive Control (red cap)	Control-DNA *H. pylori* 23S rRNA Mutant A2142G (resistant to clarithromycin)	1 x 300 µl	-25 °C to -15 °C
HPY Reaction Mix (white cap)	Reaction mix	1 x 250 µl	-25 °C to -15 °C
Nuclease-free water (PCR grade) (blue cap)	Nuclease-free water	1 x 1000 µl	-25 °C to -15 °C

Delivery and Storage
Shipment is with coolpacks. When stored properly, the kit components are stable until the specified expiration date. Store kit protected from light.
The reaction mix (white cap) should be thawed on ice. To avoid repeated thawing and freezing, the reaction mix should be aliquoted and stored at -25 °C to -15 °C.

Quality Control Release Testing
In accordance with the ISO 13485-certified Quality Management System of ingenetix, each lot is tested against predetermined specifications to ensure consistent product quality. Quality control is performed with plasmids containing parts of the pathogen DNA.

6. Additionally required materials and devices

- Reagents and devices for DNA-extraction appropriate for the sample material
- Optional: Nuclease-free water for dilution of IC
- Disposable powder-free gloves
- Pipettes (adjustable) and sterile pipette tips with filters
- Vortex Mixer
- Real-time PCR instrument which can detect fluorescence in the FAM/SYBR® Green channel and performance of a melt curve (recommended are instruments of the Quantstudio™ series(Thermo Fisher Scientific), LightCycler 480 (Roche), LightCycler® 1.1/1.2/1.5 oder 2.0 Instrument (Roche), ABI® 7500 Real-time PCR System (Thermo Fisher Scientific) or Magnetic Induction Cycler (Bio Molecular Systems)
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material

7. Precautions and safety information

7.1 General information

- For *in vitro* diagnostic use. The use of this kit is limited to qualified personnel instructed in real-time PCR and *in vitro* diagnostic procedures.
- Transportation of clinical specimens must comply with local regulations for the transport of biological materials.
- Specimens should be handled as if infectious in accordance with safe laboratory procedures. Wear protective powder-free disposable gloves when handling kit reagents and specimens.
- Discard specimens, reagents, and waste according to your local safety regulations.
- Improper collection, transport or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Quality of DNA has a profound impact on the test performance. Ensure that the used DNA extraction system is compatible with real-time PCR technology.
- The real-time PCR instrument should be calibrated, serviced and cleaned regularly.
- Protect kit components from light.
- Avoid mixing reagents of different kits and lots and check expiry date of the kit.

7.2 Specific information

A workflow must be followed to avoid false positive results due to detection of contaminating DNA.

<u>Recommended measures to avoid DNA contamination:</u>

- Use separated workspaces for specimen preparation, set-up of real-time PCR and amplification. Supplies and equipment must be dedicated to each of these separate workspaces to ensure workflow in the laboratory from pre- to post-PCR.
- Laboratory benches and devices must be cleaned regularly.
- Sample preparation should be performed in a laminar flow box. Clean laminar flow box regularly in all areas.
- If possible, leave consumables and pipettes in the laminar flow sterile bench and PCR workstation.
- Preparation of real-time PCR should be done in a PCR workstation.
- The use of sterile aerosol-resistant pipette tips is required.
- Use only DNA-free consumables.
- Wear lab coat.
- Work only with powder-free disposable gloves, do not touch the outer surface of the palm and fingers of the gloves when putting them on. Change gloves frequently. To avoid skin contact, wear gloves over the sleeves of the lab coat. Use disposable sleeve protectors if possible.
- Do not touch the rim or threads of open vials.
- Caution is advised when handling specimens and positive control to avoid cross-contamination.
- Store positive and potentially positive material separately from all other reagents.
- For a valid interpretation of results, a negative control shall be included during DNA-extraction (e.g., extraction of water instead of sample material), in to exclude false-positive results due to contamination with DNA of pathogen during extraction.
- Optional: include a negative control of PCR per PCR-run (nuclease-free water instead of sample, NTC).

8. Limitations

- Reliable results with this test are only achieved by appropriate specimen collection, transport and storage, as well as an appropriate DNA extraction procedure.
- DNA extraction and *H. pylori* detection have been validated for stool samples and biopsies with this assay.
- A negative test result does not exclude the possibility of *H. pylori* infection, because test results may be affected by improper specimen collection, technical error, specimen mix-up or pathogen quantities below the assay sensitivity. The presence of PCR inhibitors may lead to invalid results.
- For this assay highly specific primers and probes have been selected. However, false-negative or less sensitive results might be obtained due to sequence heterogeneity within the target region of not yet described clinical subtypes.
- For clarithromycin resistance testing, the three major point mutations at two positions (A2142C, A2142G, and A2143G) within the 23S rRNA most commonly associated with clarithromycin resistance in *H. pylori* isolates are detected.
- Mixed infections of wild-type and A2142G or A2142C can be detected. However, depending on the ratio, some mixed infections may not be possible to detect. It may not be possible to detect a mixed infection of wild-type and A2143G.
- Stool samples must be fresh or frozen.
- Results should be interpreted in consideration of clinical and laboratory findings.

9. Preparation of samples

BactoReal® Kit *H. pylori* ClariRes is suitable for analysis of DNA extracts from human gastric tissue biopsies and fresh or frozen stool samples without pre-enrichment.

Sample collection and storage:

- Gastric tissue biopsies can be stored in microcentrifuge tubes containing sterile physiological saline (approx. 100 µl) at 2-8 °C for up to 48 h. If it is not guaranteed that the sample will be processed within 48 h, the sample shall be immediately frozen dry without additives at -20/-80 °C.
- Stool samples: It is recommended to process samples immediately after collection. Store samples at 2-8 °C for no longer than 48 hours or freeze at -20/-80 °C in microcentrifuge tubes.

Purified DNA should be stored at -25 to -15 °C.

Extract samples with a DNA extraction system compatible with real-time PCR technology and appropriate for the sample material.

- For manual extraction recommended: QIAamp Fast DNA Stool Mini Kit (Qiagen) for stool samples and QIAamp DNA Mini Kit (Qiagen) for biopsies.
- For automated extraction recommended: MagNA Pure 24 Total NA Isolation Kit (Roche) with the MagNA Pure 24 System (Roche) for stool samples.

Depending on the protocol of the selected manufacturer, use the indicated amount of sample for purification and perform DNA extraction according to the instructions.
The test was validated with 100 mg stool as starting material. Using too small an amount of stool sample may affect the sensitivity of the test. Using too large an amount of stool sample or biopsy material may inhibit the test. In case of inhibition, the sample must be diluted 1:5 to 1:10.

Note: Melting temperatures (Table 1, Table 2) of wild type and mutants may change depending on the extraction method. When using extraction methods not recommended by ingenetix, an evaluation of the extraction method with an H. pylori wild type and the mutants must be performed. This can be done, for example, with the DNA eluate of an H. pylori negative sample spiked with the positive controls.
Always include an extraction negative control during DNA-extraction (e.g., extraction of water instead of sample material).

Quality control for DNA extraction and PCR inhibition
The DNA IPC system (internal DNA positive control) is used as a control for DNA extraction, identifies possible PCR inhibition and confirms the integrity of kit reagents.
An artificial target DNA (*H. pylori* Internal Positive Control, IC) is added during extraction.
For control of DNA extraction, the undiluted IC must be added directly to the lysis buffer (or added to the sample after the lysis buffer has been added to the sample):

→ Per sample, add 1 µl IC (orange cap)

Note: The undiluted IC shall not be added to sample material in the absence of lysis buffer, as degradation may occur. It must be added to the lysis buffer.
If the IC has not been added during extraction, it can be added at a later stage to the PCR master mix as quality control for the PCR reaction. In this case, freshly dilute the IC 1:100 with nuclease-free water and add 1 µl of the dilution/PCR reaction.
Caution: The IC shall not be added to the master mix undiluted.

10. Preparation of real-time PCR

- Include one positive control (red cap), one extraction negative control and optional one negative control (nuclease-free water) per PCR run.
- It is generally recommended to analyse samples in duplicates, which increases the probability of detection of *H. pylori* in stool samples.
- Thaw DNA samples on ice.
- Kit components, such as assay, IC, and water, must thaw completely at room temperature before preparing the master mix. Thaw the reaction mix on ice, mix gently to ensure a homogeneous solution and keep on ice until the master mix is prepared. After thawing, gently mix the components and briefly centrifuge at low speed. All reagents should be completely thawed and mixed well before starting the test. It is recommended to aliquot the HPY Reaction Mix to avoid several freeze/thaw cycles.
- **Sample**
  → Use 2 µl of DNA extract from stool samples or 5 µl of DNA extract from biopsies.
- **Positive Control**
  → Always pipette the positive control last. For the positive control, use 2 µl or 5 µl of H. pylori Wild-type PC or 2 µl or 5 µl of H. pylori A2142G PC.
- **Negative Control of PCR**
  → For the negative control use 2 µl or 5 µl of Nuclease-free water.

10.1. Pipetting scheme

Please use the following pipetting scheme if IC is added to extraction:

		Per sample (biopsies)	Per sample (stool)
Preparation of Master Mix (mix well)	Nuclease-free water	9.0 µl	12.0 µl
	HPY Reaction Mix	5.0 µl	5.0 µl
	H. pylori Assay Mix	1.0 µl	1,0 µl
	Total volume	**15.0 µl**	**18.0 µl**
Preparation of PCR	Master mix	15.0 µl	18.0 µl
	Sample	5.0 µl	2.0 µl
	Total volume	**20.0 µl**	**20.0 µl**

Please use the following pipetting scheme if IC is added to the PCR reaction:

		Per sample (biopsies)	Per sample (stool)
Preparation of Master Mix (mix well)	Nuclease-free water	8.0 µl	11.0 µl
	HPY Reaction Mix	5.0 µl	5.0 µl
	H. pylori Assay Mix	1.0 µl	1.0 µl
	H. pylori ClariRes Internal Positive Control; freshly diluted 1:100	1.0 µl	1.0 µl
	Total volume	**15.0 µl**	**18.0 µl**
Preparation of PCR assay	Master mix	15.0 µl	18.0 µl
	Sample	5.0 µl	2.0 µl
	Total volume	**20.0 µl**	**20.0 µl**

→ **If the IC was not added during extraction:** Freshly dilute the IC (orange cap) 1:100 with nuclease-free water and add 1 µl per sample directly to the master mix. In this case, the IC is used for quality control of the PCR reaction.

- Prepare the Master Mix according to the number of samples, calculating an additional volume of approx. 10% to account for pipetting loss.
- Pipette 15 µl (for biopsy samples) or 18 µl (for stool samples) of the prepared Master Mix per sample into the well of the optical reaction plate.
- Then add 5 µl (for biopsy samples) or 2 µl (for stool samples) of the extracted sample or controls. Pipette the positive control at last.
- Seal the plate with a suitable optical sealing material.
- Vortex the sealed plate for 1-2 seconds and briefly centrifuge the plate.

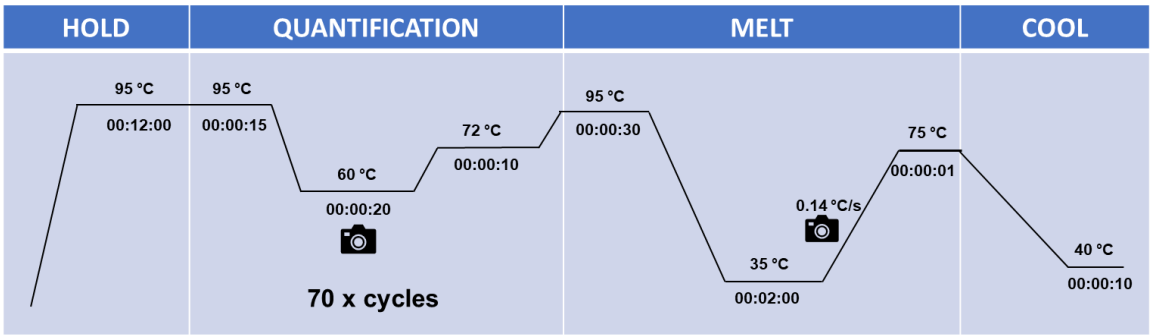
10.2. Programming of temperature profile

Please find further information on programming the instruments in the respective operator's manuals.

<u>Select detection channels:</u>

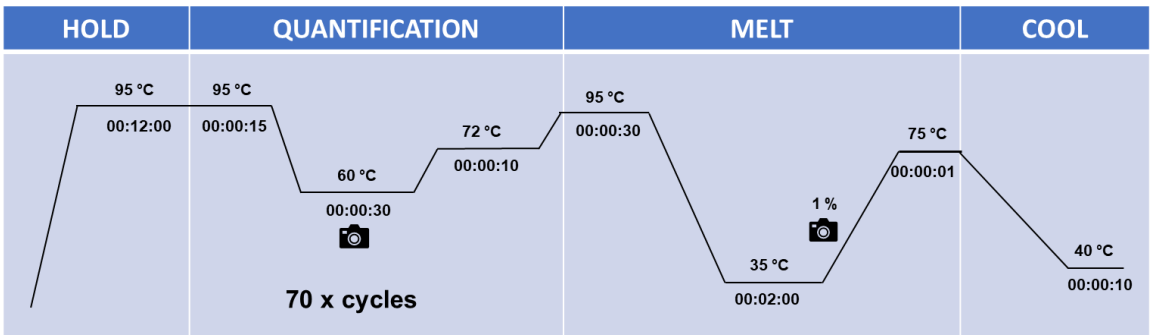
Quantstudio™ (Thermo Fisher Scientific):

Target = SYBR/NONE
Passive Reference = NONE



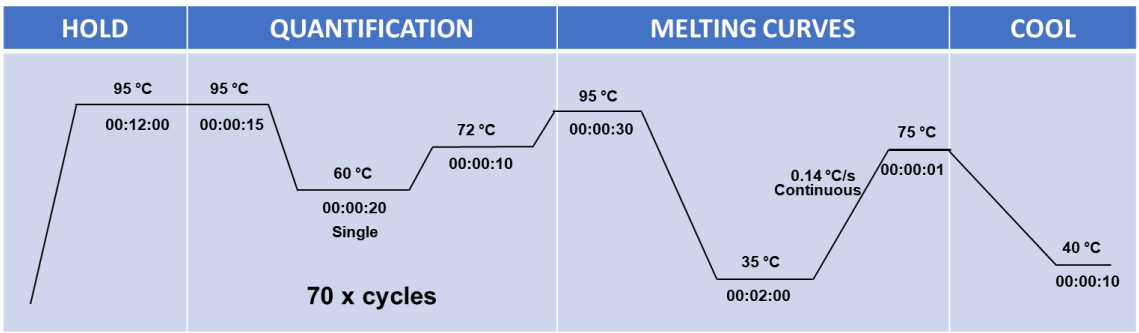
ABI® 7500 (Thermo Fisher Scientific):

Target = SYBR/NONE
Passive Reference = NONE



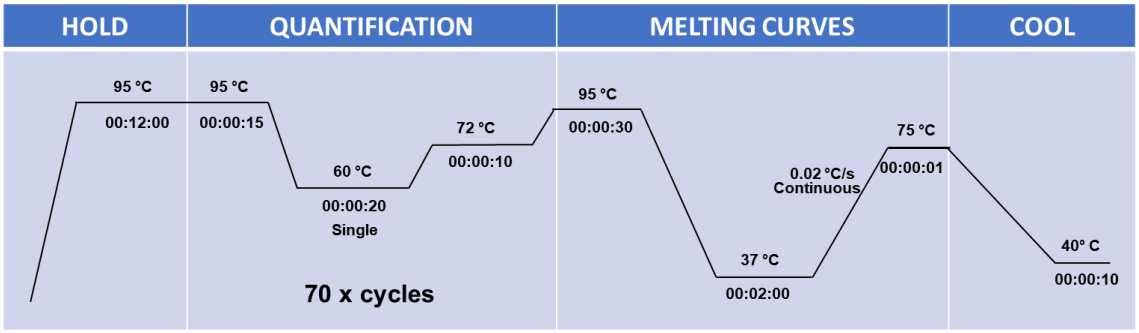
LightCycler® 480 (Roche):

Target = SYBR Green I /HRM dye



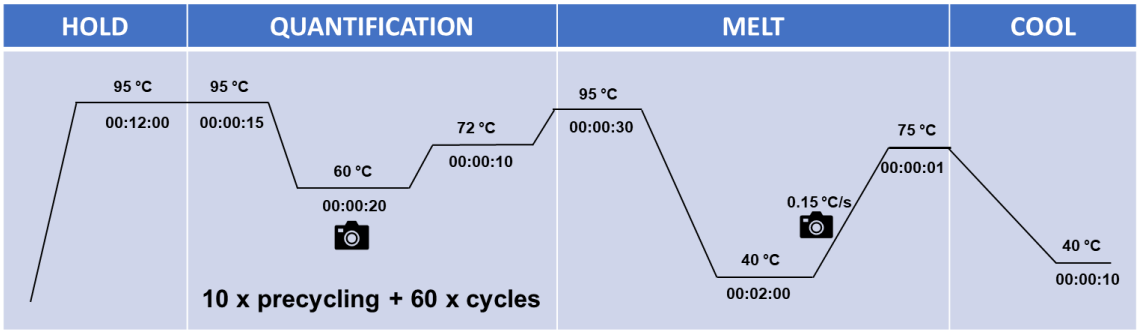
LightCycler® 2.0 (Roche):

Fluoreszenzkanal 530 nm



MIC (Bio Molecular Systems):

Target: Green



11. Interpretation of PCR-data

For the analysis of the PCR results, select the fluorescence display options FAM/SYBR® Green channel.

Interpretation of the results is based on melt curve analysis. The Cq values of the amplification curve are different for wild type and mutants and should only be used as additional information but not for the interpretation of the results.
Assessment of clinical specimen test results should be performed after the positive and negative controls and the IC have been examined and determined to be valid. If results of controls are not valid, no interpretation of results with clinical samples is possible.

Table 1 shows the criteria for valid positive and negative controls. Table 2 shows interpretation of data with clinical samples.

Table 1 Interpretation of controls

	Melting temperature Tm*	**Interpretation**
Negative control of extraction	49-51 °C	Valid
Optional: Negative control of PCR (NTC)	49-51 °C	Valid
H. pylori wild-type positive control	62-65 °C	Valid
H. pylori A2142G mutant positive control	58-61 °C	Valid

* exact Tm depending on instrument used

Once analysis is completed, the following results are possible:

Table 2 Interpretation of data with clinical samples

Melting temperature (Tm)	**Detection of**	**Interpretation**
49-51 °C	Internal positive control (IC)	No inhibition of PCR-reaction
62-65 °C	Wild-type	Sample contains *H. pylori* (Clarithromycin susceptible strain)
57-59 °C	Mutant A2142C	Sample contains *H. pylori* (Clarithromycin resistant strain)
58-61 °C	Mutant A2142G	Sample contains *H. pylori* (Clarithromycin resistant strain)
59-61 °C	Mutante A2143G	Sample contains *H. pylori* (Clarithromycin resistant strain)

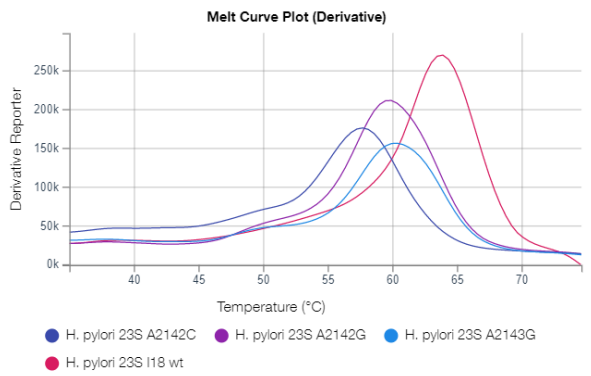
In case of detection of *H. pylori*, the detection of the IC is not essential, since high concentrations of *H. pylori* DNA can lead to a reduced or absent fluorescence signal of the internal positive control (competition of PCR).

Always perform analyses of stool samples in duplicates. In cases where only one out of two reactions is positive for *H. pylori,* the sample should be considered as positive. In some patients, a double infection with the wild type and a mutant may be present. In this case (Figure 1 G, H), the formation of a double peak in the melting curve occurs.

In case of invalid data, analysis has to be repeated with the remaining or newly extracted DNA sample. Information about possible sources of error and their solution can be found in 12. Troubleshooting.

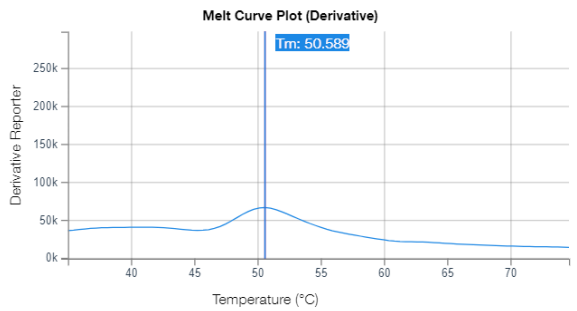
Results for *H. pylori* wild-type or mutated sequences and internal positive control (IC)

A



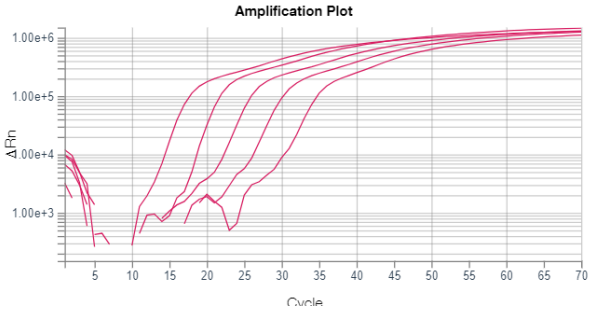
***H. pylori* melting curves – Quantstudio™ 7 Pro**

B



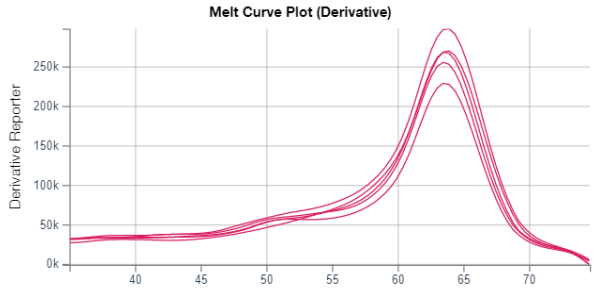
IC melting curve - Quantstudio™ 7 Pro

C



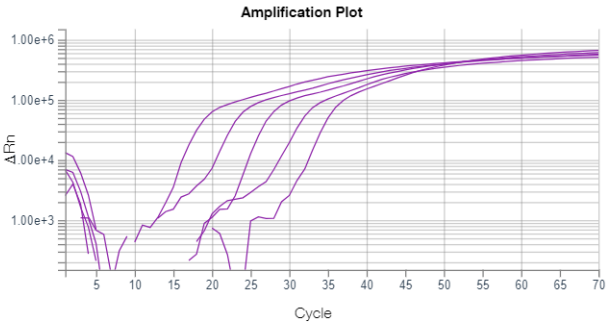
***H. pylori* Wildtyp - amplification Quantstudio™ 7 Pro**

D



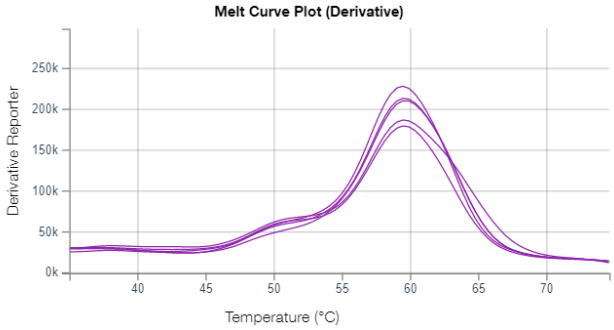
***H. pylori* Wildtyp – melting curve Quantstudio™ 7 Pro**

E



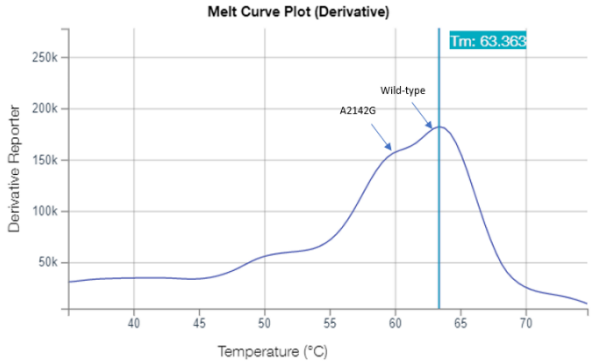
***H. pylori* A2142G - amplification Quantstudio™ 7 Pro**

F



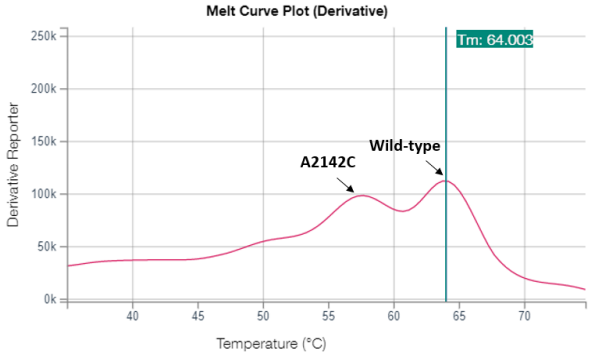
***H. pylori* A2142G – melting curve Quantstudio™ 7 Pro**

G



H. pylori wild type + A2142G melting curves at a 1:10 mixing ratio

H



H. pylori wild type + A2142C melting curves at a 1:10 mixing ratio

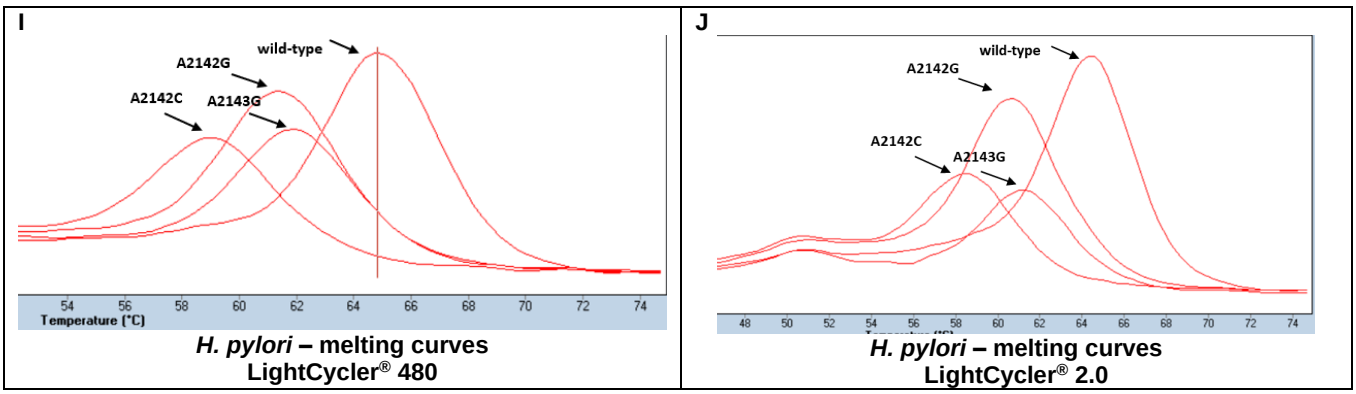


Fig. 1 Amplification and melt curves obtained with *H. pylori* DNA

12. Troubleshooting

12.1. No *H. pylori* specific signal with positive controls

- Incorrect programming of the temperature profile of the PCR instrument.
  → Compare the temperature profile with the protocol. Please note, that PCR requires **70 cycles**.
- Incorrect configuration of the PCR reaction.
  → Check your work steps by means of the pipetting scheme (see preparation of real-time PCR) and repeat the PCR, if necessary.

12.2. No signal with IC and no *H. pylori* specific signal with clinical sample

- The PCR reaction was inhibited. No diagnostic statement can be made.
  → Make sure that you use a recommended method for DNA isolation and stick closely to the manufacturer's instructions.
  → If no operating mistakes during extractions can be retraced, it is recommended to repeat the PCR with lower amounts of DNA-eluate (1/5 or 1/10 of sample volume + the adequate amount of H_2O).
- Incorrect PCR conditions.
  → Check the PCR conditions and repeat the PCR, if necessary.

12.3. No peak separation due to mixed infection (Fig. 1 G, H)

- Possibly the software does not discriminate between two peaks
  → Analyse the peaks in the graphical display

12.4. Signal between 57 und 65°C in the negative control

- A contamination occurred during preparation of the PCR.
  → Repeat the PCR with new reagents in replicates.
  → Strictly pipette the positive controls at last.
  → Make sure that work space and instruments are decontaminated at regular intervals.

12.5. Signal between 57 und 65°C in the negative control of DNA-extraction

- A contamination occurred during extraction.
  → Repeat the extraction and PCR using new reagents.
  → Make sure that work space and instruments are decontaminated at regular intervals.

13. Specification

13.1. Analytical sensitivity – Limit of detection (LoD95%)

The analytical sensitivity (defined as the smallest amount of DNA that can be detected) is 10 target copies/reaction for wild-type *H. pylori* which corresponds to 5 fg *H. pylori* DNA/PCR.
The LoD95% (defined as the concentration where 95% of the repeats were positive) is 16 target copies/reaction for wild-type *H. pylori* which corresponds to 8 fg *H. pylori* DNA/PCR. With mutant sequences, sensitivity can be 3- to 10-fold lower.

13.2. Analytical specificity

Method BLAST analysis: The specificity is ensured by the selection of specific primers and probes. The primers and probes were checked for possible homologies to published sequences by sequence comparison analyses using the Basic Local Alignment Search Tool (BLAST). This also ensured the detection of all so far known *H. pylori* strains.

Result: By *in-silico* analysis, a 100% cross-reactivity with *H. fennelliae, H. heilmanii, H. acinonychis* and *H. cetorum* was identified. *Helicobacter fennelliae* can cause gastroenteritis, proctitis and bacteremia, while *H. heilmanii* can cause chronic gastritis in humans. *Helicobacter acinonychis* and *H. cetorum* have no clinical relevance for humans.

Method testing exclusivity:
Analytical specificity was determined by testing against genomic DNA from various bacteria, including *Campylobacter jejuni, Corynebacterium diphtheria, Listeria inocua, Listeria monocytogenes, Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcous aureus, Salmonella enterica, Salmonella typhinorum, Bordetella pertussis*, *E. coli* O157 and *Neisseria meningitidis*.

Result: There was no cross-reactivity observed.

13.3. Precision

The intra-assay variation of the Tm was less than 0.2%, the inter-assay variation less than 0.5% and the inter-lot variation less than 0.7%.

13.4. Diagnostic evaluation

Method: Diagnostic evaluation of BactoReal® Kit *H. pylori* ClariRes was performed by an exernal service provider. A total of 100 DNA extracts from gastric biopsies were included in the study. These contained 50 samples of *H. pylori* wild-type and 40 samples of *H. pylori* mutants as well as 10 negative samples. The DNA was extracted using QIAamp® DNA Mini Kit (Qiagen). For comparison, a reference method accredited according to EN ISO 15189 was used. PCR was performed using a LightCycler® 480 II System.

Result: All samples were correcly identified. The specificity and sensitivity of the test with regard to detection of *H. pylori* and clarithromycin resistance was 100%. See Table 3 - Table 6.

Table 3 Results from 100 tested clinical isolates (gastric biopsies) 2×2 contingency table

	Reference			
		pos	neg	Total
BactoReal® Kit *H. pylori* ClariRes	pos	90	0	90
	neg	0	10	10
	Total	90	10	100

One sample showed a discordant result. It was negative with the reference method, but positive with BactoReal® Kit *H. pylori* ClariRes and positive with a second reference method. A repeat experiment with BactoReal® Kit *H. pylori* ClariRes gave a negative result, indicating that this sample contained levels of *H. pylori* DNA at the detection limit.

Table 4 Statistical evaluation of the diagnostic validation (gastric biopsies)

	Value	95% CI
Sensitivity	100.00%	95.98% to 100.00%
Specificity	100.00%	69.15% to 100.00%
Positive Predictive Value (*)	100.00%	
Negative Predictive Value (*)	100.00%	
Prevalence	90.00%	

In addition, 28 stool samples were spiked with various amounts of wild-type *H. pylori* or A2143G *H. pylori* mutant strains. As control, 10 negative stool samples were included. A total of 15 samples spiked with wild-type *H. pylori* and detected by two reference methods and *H. pylori* ClariRes Assay (RTGM100), were also positive BactoReal Kit® *H. pylori* ClariRes. Approximately 20 genome copies could be detected. From 13 samples spiked with A2143G *H. pylori* mutant strain and detected by the two reference methods, 12 were correctly identified by BactoReal Kit® *H. pylori* ClariRes. At least, 200 genome copies per reaction could be detected. The negative stool extracts did not show any unspecific signal.

Table 5 Results from 38 isolates (stool samples), 2 x 2 contingency table

	Reference			
		pos	neg	Total
BactoReal® Kit *H. pylori* ClariRes	pos	27	0	27
	neg	1	10	11
	Total	28	10	38

Table 6 Statistical evaluation of the diagnostic validation (stool samples)

	Value	95% CI
Sensitivity	96.43%	81.65% to 99.91%
Specificity	100.00%	69.15% to 100.00%
NPV	90.91%	
PPV	100.00%	
Prevalence	73.68%	

14. Revision history

Revision	Date	Description
1.1en	01 May 2023	**Change of the company address** **7. Precautions and safety instructions** Update of this section Inclusion of additional information on specimen collection and storage, processing of specimens after collection, storage conditions of specimens, transport and storage conditions of purified DNA. Recommended measures to avoid DNA contamination added. **9. Preparation of samples** Note added, that extraction methods not recommended by ingenetix must be validated with H. pylori wildtype and mutant.

Note:
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Technical support
ingenetix GmbH, Haidingergasse 1, 1030 Vienna, Austria
Telephone: +43 (0)1 36 198 01; **E-Mail:** office@ingenetix.com