BactoReal® Kit *H. pylori* ClariRes

Kit version 1.0

For *in vitro* diagnostic use only

BactoReal® Kit *H. pylori* ClariRes			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUB1000	50	SYBR® Green channel	SYBR® Green channel

Kit contents:

- Detection assay for *H. pylori* and for internal positive control (control of PCR amplification and DNA extraction)
- Target for internal positive control
- Reaction mix
- Nuclease-free water
- Positive controls for *H. pylori* wild type and mutant

Background: *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 23S rRNA gene of *H. pylori*.

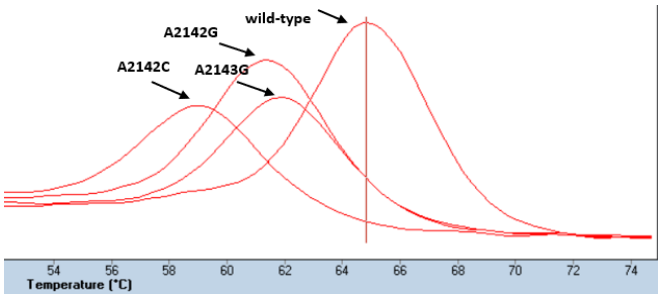
Intended pupose: BactoReal® Kit *H. pylori* ClariRes is a non-automated CE-certified IVD test, based on real-time polymerase chain reaction (PCR), for the qualitative detection of DNA (23S rRNA gene) of *Helicobacter pylori* (*H. pylori*) in combination with the detection of a possible clarithromycin 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.
The wild-type sequence and the mutants are detected and distinguished by melting curve analysis in the FAM/SYBR® Green channel following amplification. 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.
PCR-platforms: BactoReal® Kit *H. pylori* ClariRes is compatible with the real-time PCR instruments LightCycler® 1.1/1.2/1.5 or 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).

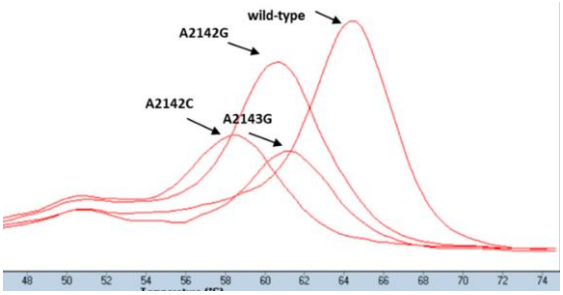
Performance data: The LoD95% (defined as the concentration where 95% of the repeats were positive) is 16 target copies/reaction for wild-type *H. pylori,* which corresponds to 8 fg *H. pylori* DNA/PCR. With mutant sequences, sensitivity can be 3- to 10-fold lower.
By *in-silico* analysis, a 100% cross-reactivity with *H. fennelliae, H. heilmanii, H. acinonychis* and *H. cetorum* was identified.
Diagnostic evaluation was performed with 100 DNA extracts from gastric biopsies. Samples contained 50 samples of *H. pylori* wild-type and 40 samples of *H. pylori* mutants as well as 10 negative samples (Table 1).
In addition, 28 stool samples spiked with various amounts of *H. pylori* wild-type or *H. pylori* mutant A2143G strains were tested. Approximately 20 genome copies of wild-type and 200 genome copies of *H. pylori* mutant A2143G per PCR reaction could be detected. As control, 10 negative stool samples were included. The negative stool extracts did not show any unspecific signal.

Table 1 Results of clinical validation with gastric biopsies

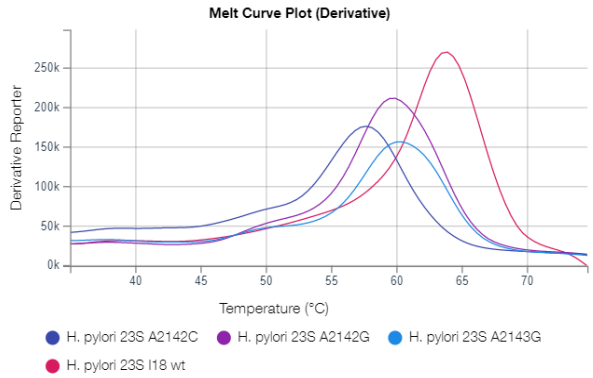
	Value	95% CI
Sensitivity	100.00%	95.98% to 100.00%
Specificity	100.00%	69.15% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Prevalence	90.00%	



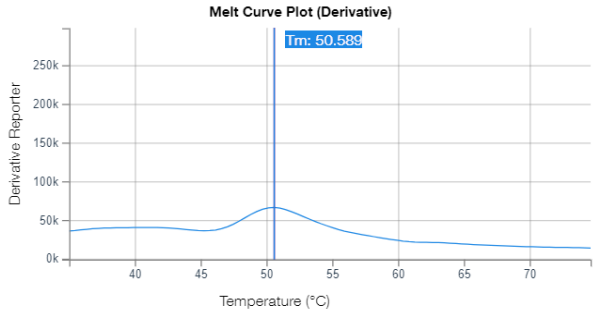
Melting curves of *H. pylori* LightCycler® 480



Melting curves of *H. pylori* LightCycler® 2.0



Melting curves of *H. pylori* Quantstudio™ 7 Pro



Melting curve of internal positive control Quantstudio™ 7 Pro

Figure 1 Performance of BactoReal® Kit *H. pylori* ClariRes