H. pylori ClariRes Assay

Product Name: *H. pylori* ClariRes Assay

Order No.: RTGM100

Unit: 50 reactions

Background: A non-invasive *H. pylori* detection in combination with clarithromycin susceptibility testing from stool samples provides a convenient monitoring of a *H. pylori* infection for the patient.
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 of *H. pylori*.

PCR-platforms: *H. pylori* ClariRes Assay is developed and validated for the LightCycler® 1.1/1.2/1.5/2.0 instruments (Roche).

Kit content: *H. pylori* ClariRes Assay contains primers and a fluorescence-labeled probe for testing up to 50 samples. Furthermore, it contains an internal positive control to verify the absence of PCR-inhibitors for reliable negative results, two positive control samples for *H. pylori* (wild-type and one mutant), water as negative control and a manual. The amplification mix is not included. Store kit components at -20°C and protect from light.
Additional required reagents (not included): LC™-FastStart DNA Master SYBR® Green I (Roche Diagnostics order no. 12239264001; kit for 480 reactions of 20 µl final reaction volume, or order no. 03003230001; kit for 96 reactions of 20 µl final reaction volume).

Description: *H. pylori* ClariRes Assay is an ivD-certified test for the simultaneous detection of *H. pylori* and of the three major point mutations in the 23S rRNA gene associated with clarithromycin resistance in *H. pylori* isolates. The three point mutations and the wild-type-sequence of the 23S rRNA gene of *H. pylori* are amplified in one PCR reaction and are subsequently differentiated by melting curve analyses at 670 nm (F3). A melting temperature of 63°C identifies the wild-type sequence of the 23S rRNA of *H. pylori*. A melting temperature of 58°C clearly indicates the mutant A2142C, while a melting temperature of 54°C indicates the presence of the mutants A2142G or A2143G. The presence of mixed infections results in two melting temperatures and can be interpreted up to a ratio of 1:10. A melting temperature of 47°C identifies the internal positive control.

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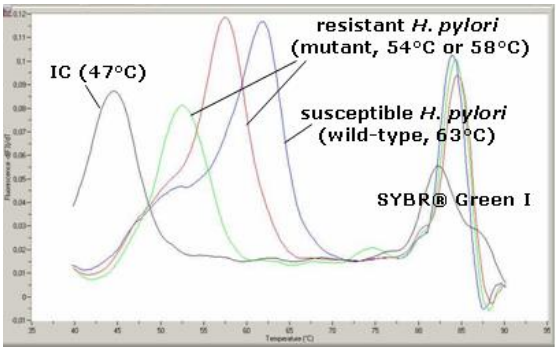
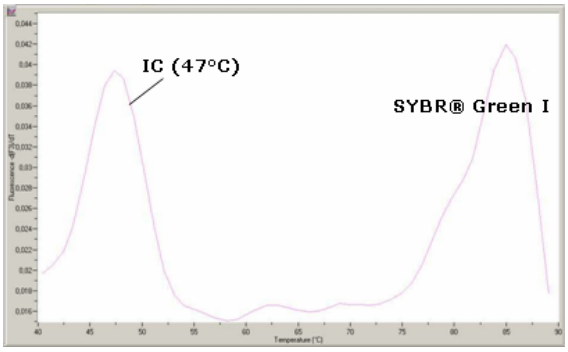
H. pylori ClariRes Assay allows the examination of DNA freshly extracted from or frozen stool or biopsies without pre-enrichment.

Specificity and sensitivity: *H. pylori* ClariRes Assay has an analytical sensitivity (defined as the smallest amount of DNA that can be detected) of 0.5 fg *H. pylori* DNA/PCR (equal to 0.3 CFU). The LoD 95% (defined as the concentration, where 95% of 20 PCR repeats were positive) is 5 fg *H. pylori* DNA/PCR (equal to 3 CFU). Therefore, 5 fg/PCR-reaction can be detected with a probability of 95%.
Regarding the detection of the clarythromycin resistance, the specificity is 100% both for stool and biopsy samples, the sensitivity is 73% for stool and 82% for biopsies. Regarding the detection of *H. pylori*, the test has a specificity of 98% (both for stool and biopsy samples) and a sensitivity of 98% for stool and 100% for biopsies (Schabereiter-Gurtner et al., 2007).
The test reveals 100% cross-reactivity with *H. fennelliae, H. heilmanii, H. acinonychis* and *H. cetorum. 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. The poor cross-reactivity with other non-pylori *Helicobacter* species is assumed to have no diagnostic relevance, due to poor PCR amplification even in high DNA concentrations thereof.



LightCycler® 1.5: channel F3
Melting curves of *H. pylori* clarithromycin resistant (mutant), clarithromycin susceptible (wild-type) strains and of the internal positive control (IC)



LightCycler® 1.5: channel F3
Melting curve of the internal positive control (IC)

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.

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