RIDA®GENE Helicobacter pylori
real-time PCR

Art. Nr.: PG2305
100 Reactions

For \textit{in-vitro} diagnostic use.

\( -20 \, ^\circ \text{C} \)

R-Biopharm AG, An der neuen Bergstraße 17, D-64297 Darmstadt, Germany
Tel.: +49 (0) 61 51 81 02-0 / Telefax: +49 (0) 61 51 81 02-20
1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Helicobacter pylori is a real-time PCR for the direct, qualitative detection of *Helicobacter pylori* and its resistance to clarithromycin from human native tissue biopsy material.

The RIDA®GENE Helicobacter pylori real-time PCR is intended for use as an aid in diagnosis of gastric infections caused by *Helicobacter pylori*.

2. Summary and Explanation of the test

*Helicobacter pylori* (*H. pylori*) is a gram-negative rod-shaped bacterium which colonises the human gut. *H. Pylori* increases the secretion of stomach acid and hence leads to different gastric infections such as Type B Gastritis, gastric ulcers or duodenal ulcers. Worldwide, *H. pylori* has a prevalence rate of 50 %, whereas the infection rate is higher in developing countries compared to developed countries. In Germany, about 33 Mio People are infected with *H. pylori* of which 10-20 % develop ulcers. While the *H. pylori* strain type 2 lacks the pathogenicity factors cag and VacA, an infection with *H. pylori* strain type 1 leads to gastroduodenal ulcers and in case of a chronic infection, significantly increases the risk of gastric cancer. To protect itself from gastric acid, *H. pylori* settles inside the gastric mucosa. Here, *H. pylori* splits urea by the enzyme urease to increase the pH value in its close surroundings.

Today, *H. pylori* is detected by microscopy or by using the helicobacter-urease assay from gastric biopsies. Other detection methods are antigen testing or breath tests.

After diagnosis of *H. pylori*, different treatment measures are possible. Often, the “Triple Therapy” is used which consists of a combination of Amoxicillin, Clarithromycin and a proton pump inhibitor or Metronidazol, Clarithromycin and a proton pump inhibitor. However, increasing clarithromycin resistance lowers the success rate of such a treatment by 30 %. Also, other more and more often occurring resistances against antibiotics such as Metronidazol or Levofloxacin (Fluoroquinolone) lead to higher failure in *H. pylori* eradication therapies.

3. Test principle

The RIDA®GENE Helicobacter pylori is a real-time PCR for the direct, qualitative detection of Helicobacter pylori from human biopsy samples.

After DNA-isolation, amplification of gene fragments (16S rRNA and 23S rRNA, if present) specific for *Helicobacter pylori* and a potential resistance to clarithromycin occurs.
The amplified target for *Helicobacter pylori* is 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 fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE Helicobacter pylori assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and/or to determine possible PCR inhibition.

4. Reagents provided

Tab. 1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

<table>
<thead>
<tr>
<th>Kit Code</th>
<th>Reagent</th>
<th>Amount</th>
<th>Lid Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reaction Mix</td>
<td>2x 1100 µl</td>
<td>yellow</td>
</tr>
<tr>
<td>2</td>
<td>Taq-Polymerase</td>
<td>1x 11 µl</td>
<td>red</td>
</tr>
<tr>
<td>D</td>
<td>Internal Control DNA</td>
<td>2x 1800 µl</td>
<td>orange</td>
</tr>
<tr>
<td>N</td>
<td>PCR Water</td>
<td>1x 500 µl</td>
<td>white</td>
</tr>
<tr>
<td>P</td>
<td>Positive Control</td>
<td>1x 200 µl</td>
<td>blue</td>
</tr>
</tbody>
</table>

5. 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 and fully defrost reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Reagents can sustain up to 5 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all reagents should be stored cold in an appropriate way (2 - 8 °C).
6. Additional necessary reagents and necessary equipment

- The RIDA®GENE Helicobacter pylori real-time PCR Assay is suitable for use with following extraction platforms and real-time PCR instruments:
  - Extraction platform:
    MagNA Pure 96 (Roche)
  - Real-time PCR instrument:
    Roche: LightCycler® 480
    Agilent Technologies: Mx3005P
    Applied Biosystems: ABI 7500
    Abbott: m2000rt
    Bio-Rad: CFX96™
    Cepheid: SmartCycler®
    QIAGEN: Rotor-Gene Q

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit I (PG0001) for use with the LightCycler® 480
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves

7. Precautions for users

For in vitro diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. 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.

For more details see Material Safety Data Sheets (MSDS) at www.r-biopharm.com

8. Collection and Storage of Samples

8.1 Sample Preparation

For DNA isolation of human biopsy samples, use a commercially available DNA extraction system (e.g. MagNA Pure 96 (Roche)). Extract DNA according to the manufacturer’s instructions.

We recommend to extract the biopsie material over night at 55 °C using Proteinase K. From this sample, use the appropriate volume according to the manufacturer’s instructions.

The RIDA®GENE Helicobacter pylori real-time PCR kit contains an internal control (ICD) that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient.

If the ICD is used only as a PCR inhibition control, 1 µl of the ICD should be added to the Master-Mix (see Tab. 3).

If the ICD is used as an extraction control for the sample preparation procedure and as PCR inhibition control, 20 µl of the ICD has to be added during extraction procedure. The ICD should always be added to the specimen-lysis buffer mixture and not directly to the specimen.
9. Test procedure

9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and negative control must be included in each assay run.

We recommend to calculate an additional volume of 10% to compensate imprecise pipetting (see Tab. 2, Tab. 3). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Taq-Polymerase, the Positive Control, the PCR Water and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab. 2: Calculation and pipetting example for 10 reactions of the Master-Mix

(ICS as extraction and PCR inhibition control)

<table>
<thead>
<tr>
<th>Kit code</th>
<th>Master-Mix components</th>
<th>Volume per reaction</th>
<th>10 reactions (10% extra)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reaction Mix</td>
<td>19.9 µl</td>
<td>218.9 µl</td>
</tr>
<tr>
<td>2</td>
<td>Taq-Polymerase</td>
<td>0.1 µl</td>
<td>1.1 µl</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20.0 µl</td>
<td>220 µl</td>
</tr>
</tbody>
</table>

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

Tab. 3: Calculation and pipetting example for 10 reactions of the Master-Mix

(ICS only as PCR inhibition control)

<table>
<thead>
<tr>
<th>Kit Code</th>
<th>Master-Mix components</th>
<th>Volume per reaction</th>
<th>10 reactions (10% extra)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reaction Mix</td>
<td>19.9 µl</td>
<td>218.9 µl</td>
</tr>
<tr>
<td>2</td>
<td>Taq-Polymerase</td>
<td>0.1 µl</td>
<td>1.1 µl</td>
</tr>
<tr>
<td>D</td>
<td>Internal Control DNA</td>
<td>1.0 µl</td>
<td>11 µl</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>21.0 µl</td>
<td>231.0 µl</td>
</tr>
</tbody>
</table>

Mix the components of the Master-Mix gently and briefly spin down.
9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

**Negative control:** Add 5 µl [PCR Water](#) as negative control to the pre-pipetted Master-Mix.

*Note:* If the ICD is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the ICD to the negative control PCR-Mix.

**Sample:** Add 5 µl DNA extract to the pre-pipetted Master-Mix.

**Positive control:** Add 5 µl [Positive Control](#) to the pre-pipetted Master-Mix.

*Note:* If the ICD is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the ICD to the positive control PCR-Mix.

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument Set-up (see Tab.4, Tab. 5).

9.3 PCR Instrument Set-up

Tab. 4: Real-time PCR profile for LightCycler® 480, SmartCycler® and Rotor-Gene Q

<table>
<thead>
<tr>
<th>Initial Denaturation</th>
<th>1 min, 95 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cycles</strong></td>
<td><strong>Denaturation</strong></td>
</tr>
<tr>
<td><strong>PCR</strong></td>
<td><strong>Annealing/Extension</strong></td>
</tr>
<tr>
<td><strong>Temperature Transition Rate / Ramp Rate</strong></td>
<td><strong>Maximum</strong></td>
</tr>
</tbody>
</table>

*Note:* Annealing and Extension occur in the same step.

*Note:* Check that the “Manual Thres. Fluor Units” for Channel 1 is set to 10.0 and for Channel 2 and 4 is set to 5.0 on the SmartCycler® (Cepheid). Due to variations between different cyclers, it may be required to individually adapt the “Manual Thres. Fluor Units” for channel 1.
Tab. 5: Real-time PCR profile for Mx3005P, ABI 7500, m2000rt and CFX96™

<table>
<thead>
<tr>
<th>Initial Denaturation</th>
<th>1 min, 95 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>45 Cycles</td>
</tr>
<tr>
<td>PCR</td>
<td>15 sec, 95 °C</td>
</tr>
<tr>
<td>Denaturation</td>
<td>30 sec, 60 °C</td>
</tr>
<tr>
<td>Annealing/Extension</td>
<td></td>
</tr>
<tr>
<td>Temperature Transition Rate / Ramp Rate</td>
<td>Maximum</td>
</tr>
</tbody>
</table>

**Note:** Annealing and Extension occur in the same step.
9.4 Detection Channel Set-up

Tab. 6: Selection of appropriate detection channels

<table>
<thead>
<tr>
<th>Real-time PCR Gerät</th>
<th>Nachweis</th>
<th>Detektionskanal</th>
<th>Bemerkung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche LightCycler® 480II</td>
<td>H. pylori</td>
<td>465/510</td>
<td>RIDA®GENE Color Compensation Kit I (PG0001) is required</td>
</tr>
<tr>
<td></td>
<td>ICD</td>
<td>533/580</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clarithromycin resistance</td>
<td>618/660</td>
<td></td>
</tr>
<tr>
<td>Cepheid SmartCycler®</td>
<td>H. pylori</td>
<td>Kanal 1</td>
<td>Check that the “Manual Thres. Fluor Units” for Channel 1 is set to 10.0 and for Channel 2 and 4 is set to 5.0 *</td>
</tr>
<tr>
<td></td>
<td>ICD</td>
<td>Kanal 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clarithromycin resistance</td>
<td>Kanal 4</td>
<td></td>
</tr>
<tr>
<td>ABI 7500</td>
<td>H. pylori</td>
<td>FAM</td>
<td>Check that passive reference option ROX is none</td>
</tr>
<tr>
<td></td>
<td>ICD</td>
<td>VIC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clarithromycin resistance</td>
<td>Cy5</td>
<td></td>
</tr>
<tr>
<td>Abbott m2000rt</td>
<td>H. pylori</td>
<td>FAM</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ICD</td>
<td>VIC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clarithromycin resistance</td>
<td>Cy5</td>
<td></td>
</tr>
<tr>
<td>Agilent Techn. Mx3005P</td>
<td>H. pylori</td>
<td>FAM</td>
<td>Check that reference dye is none</td>
</tr>
<tr>
<td></td>
<td>ICD</td>
<td>HEX</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clarithromycin resistance</td>
<td>Cy5</td>
<td></td>
</tr>
<tr>
<td>Qiagen Rotor-Gene Q</td>
<td>H. pylori</td>
<td>Green</td>
<td>The gain settings have to be set to 5</td>
</tr>
<tr>
<td></td>
<td>ICD</td>
<td>Yellow</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clarithromycin resistance</td>
<td>Red</td>
<td></td>
</tr>
<tr>
<td>Bio-Rad CFX96™</td>
<td>H. pylori</td>
<td>FAM</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ICD</td>
<td>VIC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clarithromycin resistance</td>
<td>Cy5</td>
<td></td>
</tr>
</tbody>
</table>

* Due to variations between different cyclers, it may be required to individually adapt the “Manual Thres. Fluor Units” for channel 1.
10. 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. Positive and negative controls have to show correct results (see Table 7, Fig. 1, Fig. 2) in order to determine a VALID run.

The positive control has a concentration of $10^3$ copies/µl. In each PCR run it is used in a total amount of $5 \times 10^3$ copies.

Tab. 6: For a VALID run, the following conditions must be met:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Assay result</th>
<th>ICD Ct</th>
<th>Target Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTC</td>
<td>Positive</td>
<td>NA *1</td>
<td>See Quality Assurance Certificate</td>
</tr>
<tr>
<td>NTC</td>
<td>Negative</td>
<td>Ct &gt; 20</td>
<td>0</td>
</tr>
</tbody>
</table>

*1 No Ct value is required for the ICD to make a positive call for the positive control.

If the Positive Control (PTC) is not positive within the specified Ct range but the Negative Control is valid, prepare all new reactions using remaining purified nucleic acids and a new Positive Control.

If the Negative Control (NTC) is not negative but the Positive control is valid prepare all new reactions using remaining purified nucleic acids and a new Negative Control.

If the required criteria are not met, following items have to be checked before repeating the test:
- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure
Fig. 1: Correct run of the positive and negative control (*Helicobacter pylori*) on the LightCycler® 480II

Fig. 2: Correct run of the positive and negative control (Clarithromycin resistance) on the LightCycler® 480II
11. Result interpretation

The result interpretation is done according to Table 8.

Tab. 8: Sample interpretation

<table>
<thead>
<tr>
<th>Helicobacter pylori</th>
<th>Clarithromycin-Resistance</th>
<th>ICD</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>negative</td>
<td>positive/negative</td>
<td>H. pylori detected</td>
</tr>
<tr>
<td>positive</td>
<td>positive*</td>
<td>positive/negative</td>
<td>H. pylori and Clarithromycin-Resistance detected</td>
</tr>
<tr>
<td>negative</td>
<td>negative</td>
<td>positive</td>
<td>Target Gene not detected</td>
</tr>
<tr>
<td>negative</td>
<td>positive*</td>
<td>positive/negative</td>
<td>H. pylori not detected</td>
</tr>
<tr>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>Invalid</td>
</tr>
</tbody>
</table>

*NOTE: when using the LightCycler® 480 (Roche), 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; when using the Mx3005P, the ABI7500 (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.
12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.

2. This assay is only validated for biopsie material.

3. Inappropriate specimen collection, transport, storage and processing or a pathogen 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 detection of new variants resulting in a false negative result with the RIDA® GENE Helicobacter pylori assay.

6. As with all PCR based in vitro diagnostic 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 gene for Helicobacter pylori and/or Clarithromycin resistance.

8. In individual cases, weak cross-reactivity can occur in the Cy5 channel in presence of organisms that also carry the Clarithromycin wild-type genome.

9. In individual cases, a positive signal in the Cy5 channel can occur in presence of organisms which are not Helicobacter pylori, but also carry the Clarithromycin wild-type genome.
13. Performance characteristics

13.1 Clinical Performance

In a retrospective clinical validation study we analyzed 225 and 139 human clinical specimens with the RIDA®GENE Helicobacter pylori assay compared to culture and a second commercial PCR method in an institute in Germany.

Tab. 9: Correlation of the *Helicobacter pylori* and Clarithromycin-resistance results with the RIDA®GENE Helicobacter pylori real-time PCR and the reference method

*Helicobacter pylori*

<table>
<thead>
<tr>
<th>Gold Standard</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIDA®GENE Helicobacter pylori</td>
<td>Positive</td>
<td>115</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>110</td>
<td>225</td>
</tr>
</tbody>
</table>

Clarithromycin resistance

<table>
<thead>
<tr>
<th>Gold Standard</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIDA®GENE Helicobacter pylori</td>
<td>Positive</td>
<td>78</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>3</td>
<td>56</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>58</td>
<td>139</td>
</tr>
</tbody>
</table>
13.2 Analytical sensitivity

The RIDA®GENE Helicobacter pylori real-time PCR has a detection limit of ≥ 10 DNA copies per reaction for Helicobacter pylori (see Fig. 3, Fig. 4). For use with the SmartCycler®, the limit of detection is ≥ 500 DNA copies per reaction.

Fig. 3: Dilution series H. pylori (10⁵ – 10¹ DNA copies per µl) on the LightCycler® 480II

![Amplification Curves](image1)

Fig. 4: Dilution series Clarithromycin resistance (10⁵ – 10¹ DNA copies per µl) on the LightCycler® 480II

![Amplification Curves](image2)
The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

13.3 Analytical specificity

The analytical specificity of the RIDA®GENE Helicobacter pylori real-time PCR is specific for *Helicobacter pylori* from human biopsy samples. No cross-reaction could be detected for the following species (see Tab. 10):

Tab. 10 Cross-reactivity testing

<table>
<thead>
<tr>
<th>Species</th>
<th>Cross-reactivity</th>
<th>Species</th>
<th>Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>-</td>
<td>Campylobacter upsaliensis</td>
<td>-</td>
</tr>
<tr>
<td>Adenovirus 40, Human, Strain Dugan</td>
<td>-</td>
<td>E. coli (O6)</td>
<td>Proteus vulgaris</td>
</tr>
<tr>
<td>Adenovirus 41, Human, Strain Tak</td>
<td>-</td>
<td>E. coli (O26:H-)</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>-</td>
<td>Citrobacter freundii</td>
<td>-</td>
</tr>
<tr>
<td>Arcobacter butzleri</td>
<td>-</td>
<td>Clostridium difficile</td>
<td>-</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>-</td>
<td>Clostridium perfringens</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>-</td>
<td>Clostridium sporogenes</td>
<td>Giardia lamblia</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>-</td>
<td>Campylobacter coli</td>
<td>-</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>-</td>
<td>Campylobacter fetus subsp. Fetus</td>
<td>-</td>
</tr>
<tr>
<td>Campylobacter lari subsp. Lari</td>
<td>-</td>
<td>Campylobacter lari subsp. Lari</td>
<td>-</td>
</tr>
<tr>
<td>Adenovirus 1, Human, Strain Adenoid 71</td>
<td>-</td>
<td>Adenovirus 7, Human, Strain Gomen</td>
<td>-</td>
</tr>
</tbody>
</table>
Literature