1. Used

Helicobacter pylori Real Time PCR Kit

1.1. Warnings and Precautions

1.1.1. If you use ABI Prism 7000/7300/7500/Step One Plus; iCycler it 740Q/5;
Smart Cycler II; Bio-Rad CFX 96; Rotorgene 6000; Mx3000P/3005P; MJ-Option2/ Chromo4; LightCycler®480 Instrument

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during real-time PCR allows the detection of the accumulating product without having to re-open the reaction tube after the amplification cycle

3. Product Description

Helicobacter pylori is a Gram-negative, microaerophilic bacterium found in the stomach. It is also linked to the development of duodenal ulcers and stomach cancer. More than 50% of the global population harbor H. pylori in their upper gastrointestinal tract. H pylori is thought to have evolved to penetrate the mucoid lining of the stomach. Research has been conducted to identify the bacterial factors and the deregulated host cell pathways that are responsible for the progression to more severe disease states

Helicobacter pylori Real Time PCR Kit contains a specific ready-to-use system for the detection of Helicobacter pylori by polymerase chain reaction (PCR) in the real-time PCR system. The kit contains reagents and enzymes for the specific amplification of the target gene. Fluorescence is emitted and measured by the real-time systems optical unit during PCR. The detection of amplified Helicobacter pylori DNA fragment is performed in fluorimeter channel FAM. DNA extraction buffer is available in the kit. In addition, the kit contains a system to identify possible PCR inhibition by measuring the channel HEX/VIC/JOE fluorescence of the internal control (IC). An external positive control is added to the kit

4. Kit Contents

Ref | Type of Reagent | Presentation
---|---|---
1 | DNA Extraction Buffer | 1 vial, 1 ml
2 | Hp Reaction Mix | 1 vial, 950μl
3 | PCR Enzyme Mix | 1 vial, 12μl
4 | Molecular Grade Water | 2 vials, 1.5ml
5 | Internal Control(Internal Control) | 1 vial, 30μl
6 | Control (>10×10^3 copies/ml) | 1 vial, 30μl

Analysis sensitivity: 1×10^3 copies/ml, 1×10^4 copies/ml, 1×10^5 copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods, and other factors. If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher

Storage: 

- All reagents should be stored at -20°C. Storage at 4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit box.
- Repeated thawing and freezing (>2x) may result in loss of the reagent.
- Cool all reagents during the working steps.
- Reaction Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological catalog
- Real-time PCR system
- Trypsin Digestive Solution
- Cryo-container
- Real-time PCR reaction tubes/plates
- Microcentrifuge (for quick spin)
- Pipets (0.5 – 1000 μl)
- Sterile micropipettes
- Pipet tips
- Sterile micropipettes (for pipet tips)
- Disposable gloves, powder-free
- Biobactirial waste container

7. Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- A negative control is included in the kit.

8. Sample Collection, Storage and transport

- Collect samples in sterile tubes.
- Store samples at -80°C immediately or frozen at -20°C to -40°C for up to 3 months.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1. DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

9.1.1. Gastric mucosa sample

1) Take the patient’s gastric mucosa wound, and add 1 ml normal saline into the sample. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 100μl DNA extraction buffer and 4μl (200ng/μl) proteinase K, close the then vortex for 10 seconds, incubate the tube at 56°C for 3 hours.
3) Incubate the tube at 100°C for 5 minutes. The template can be used for PCR.

9.1.2. Nasopharyngeal swabs sample

1) Wash the swabs in 1 ml normal saline and vortex vigorously Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 100μl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge. 4) Incubate the tube for 10 minutes at 100°C. 5) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR.

9.3. Blood sample

1) Take 1 ml sample to a tube, add 50μl digestion buffer (1 ml/L Triton, 0.5 ml/L EDTA, 10% N-Lauroyl Sarcosine Sodium), incubate the tube at 37°C for 20 min, add 12μl Proteinase K and incubate at 56°C for 3 hours. 2) Centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet. 3) Add 100μl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge 4) Incubate the tube for 10 minutes at 100°C 5) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR.

9.4. Bacteria solution sample

1) Take 100-500 μl bacteria solution into a tube, centrifuge the tube at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 1 ml normal saline into the pellet, close the tube then vortex for 10 seconds. Centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet. 3) Add 100μl DNA extraction buffer, close the tube then vortex for 10 seconds, Spin down briefly in a table centrifuge 4) Incubate the tube for 10 minutes at 100°C 5) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR.

10. Quantitation

The positive control (1×10^3 copies/ml) is supplied in the kit. For performance of quantitative Real-Time PCR, Standard dilutions must prepare first as follows.

1) Take positive control (1×10^3 copies/ml) as the starting high in the first tube. Respectively pipette 30μl Molecular Grade Water into next three tubes. Do three dilutions as the following figures:

- 10^3 dilution
- 10^2 dilution
- 10^1 dilution

To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations

Attention:

- Mix thoroughly before next transfer
- The positive control (1×10^3 copies/ml) contains high concentration of the target DNA.

Therefore, be careful during the dilution in order to avoid contamination

11. PCR Protocol

The Master mix volume for each reaction should be pipetted as follows:

- Take 12μl Reaction Mix and Enzyme Mix per reaction multiply the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample Mix completely then spin down briefly in a centrifuge.

- Pipet 25μl (2.5μl for SmartCycler®H Master) with micropipette of sterile filter tips to each of the Real-time PCR reaction plate/tubes. Separately add 4μl(2.5μl for SmartCycler®H) DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.

- Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes

- Perform the following protocol in the instrument:

1) 94°C for 2min
2) 94°C for 38cycles
3) 95°C for 15sec, 60°C for 1min

4) (Fluorescence measured at 60°C)

5) If you use ABI Prism® system, please choose “none” as passive reference and quencher

12. Quality control

- Negative control: Negative control, positive control, internal control and QS curve must be performed correctly, otherwise the sample results is invalid

13. Data Analysis and Interpretation

The following sample results are possible:

- Ct value

<table>
<thead>
<tr>
<th>Channel</th>
<th>Cq value</th>
<th>Result Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM</td>
<td>HEX/VIC/JOE</td>
<td>QS</td>
</tr>
<tr>
<td>Molecular Grade Water</td>
<td>UNDET</td>
<td>25–35</td>
</tr>
<tr>
<td>Positive Control (qualitative assay)</td>
<td>QS curve</td>
<td>Correlation coefficient of QS curve &lt; 0.98</td>
</tr>
<tr>
<td>UNDET</td>
<td>UNDET</td>
<td>25–35</td>
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</tbody>
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