HBV Genotype B&C Real Time PCR Kit

Cat. No.: HD-0006-03

For use with PE5700/ MJ-Opticon etc. Single Color Systems
Real-Time PCR system.

User Manual
For In Vitro Diagnostic Use Only
1. Intended Use
HBV genotype B&C real-time PCR kit is used for the detection of HBV genotype B&C in serum or plasma by using real time PCR systems.

Its characteristics:
High sensitivity: lower detection line $10^2$ IU/ml
High specificity: test result will be positive, only to HBV genotypes B&C.
Short operating time: 1 and a half hours totally
Good stability: kept for 12 months at $-20^\circ$C; CV≤5%;

2. Introduction
Hepatitis B virus (HBV) is one of the most common infectious diseases in the world. More than 300 million people worldwide are estimated to have chronic HBV infection. Ten percent of these patients will die as a direct consequence of persistent viral infection. At present, seven genotypes of hepatitis B virus (HBV) have been recognized (A–G). Previous studies have indicated a geographic distribution of HBV genotype; for example, genotypes A and D are prevalent in Western countries, while B and C are more frequent in Asia. In Asia, HBV genotype C (HBV/C) induces more aggressive liver disease associated with the development of cirrhosis and hepatocellular carcinoma (HCC) than HBV/B and has a lower response rate to interferon therapy compared to HBV/B. Furthermore, HBV/A is associated more frequently with chronic infection than HBV/D in Europe. Patients infected with HBV/B in Taiwan develop HCC at a much earlier age than those with HBV/C, while young adult Japanese patients (≤30 years) infected with HBV/B do not develop HCC.

3. 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 allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

4. Product Description
HBV genotype B&C real-time PCR kit contains a specific ready-to-use system for the detection of the HBV genotype B and HBV genotype C using PCR (polymerase chain reaction) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the HBV genotype B and HBV genotype C DNA fragment. Fluorescence is emitted and measured by the real time systems’ optical unit during the PCR. Detection of amplified DNA fragment of HBV genotype B&C are performed in fluorimeter channel FAM with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and serum samples are used for DNA extraction. In addition, an external HBV genotype B positive control and HBV genotype C positive control are supplied in the kit.

5. Kit Contents

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Type of reagent</th>
<th>Presentation 25rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNA Extraction Buffer</td>
<td>1 vial, 1.8ml</td>
</tr>
<tr>
<td>2</td>
<td>HBV Genotype B Reaction Mix</td>
<td>1 vial, 950μl</td>
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<tr>
<td>3</td>
<td>HBV Genotype C Reaction Mix</td>
<td>1 vial, 950μl</td>
</tr>
<tr>
<td>4</td>
<td>PCR Enzyme Mix</td>
<td>1 vial, 22μl</td>
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<tr>
<td>5</td>
<td>Molecular Grade Water</td>
<td>1 vial, 400μl</td>
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<tr>
<td>6</td>
<td>HBV Genotype B Positive Control</td>
<td>1 vial, 30μl</td>
</tr>
<tr>
<td>7</td>
<td>HBV Genotype C Positive Control</td>
<td>1 vial, 30μl</td>
</tr>
</tbody>
</table>
6. 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 label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction Mix should be stored in the dark.

7. Additionally Required Materials and Devices
- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g)
- Vortex mixer
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and Freezer
- Tube racks

8. Warnings and Precaution
Carefully read the instructions before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, and smoke in laboratory.
- Avoid aerosols

9. Sample Collection, Storage and transport
- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

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

1) Pipet 50μl blood serum to a 0.5ml tube, add 50μl DNA extraction buffer, close the tube, then vortex for 10 seconds. Spin down briefly in a table centrifuge.
2) Incubate the tube for 10 minutes at 100°C.
3) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted, and can be used for the PCR template.

**Attention:**
A. During the incubation, make sure the tube is not open, as the vapor will volatilize into the air and may cause contamination if the sample is positive.
B. The extraction sample should be used in 3 hours or store at -20°C for one month.
C. Different brand DNA extraction kits are available. You can also use your own extraction systems or the commercial kit based on the yield. For the DNA extraction, please comply with the manufacturer’s instructions.

10.2 PCR Protocol
The Master Mix volume for each reaction should be pipetted as follows:

1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with 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. *(n: the number of reaction).*
<table>
<thead>
<tr>
<th>Reaction Volume</th>
<th>Master Mix Volume</th>
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<tbody>
<tr>
<td>36µl Reaction Mix</td>
<td>36µl × (n+1)</td>
</tr>
<tr>
<td>0.4µl Enzyme Mix</td>
<td>0.4µl × (n+1)</td>
</tr>
</tbody>
</table>

Mix completely then spin down briefly in a centrifuge.

2) Pipet 36µl Master Mix B with micropipets of sterile filter tips to each Real time PCR reaction plate/tubes. Separately add 4µl DNA sample supernatant, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.

3) Pipet 36µl Master Mix C with micropipets of sterile filter tips to each Real time PCR reaction plate/tubes. Separately add 4µl DNA sample supernatant, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.

4) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.

5) Perform the following protocol in the instrument:

\[
\begin{align*}
37°C & \text{ for 2 min, 1 cycle;} \\
\downarrow & \\
94°C & \text{ for 2 min, 1 cycle;} \\
\downarrow & \\
94°C & \text{ for 10 sec, 62°C for 40 sec, 40 cycles.}
\end{align*}
\]

Fluorescence is measured at 62°C

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

11. Data Analysis and Interpretation

The samples are HBV positive and following results are possible:

1) Signals are detected in channel FAM from Master Mix B and Master Mix C. The result is positive: the sample contains DNA of HBV genotype B and genotype C.

2) A signal is detected in channel FAM from Master Mix B. The result is positive: the sample contains DNA of HBV genotype B.

3) A signal is detected in channel FAM from Master Mix C. The result is positive: the sample contains DNA of HBV genotype C.

4) No Signals are detected in channel FAM. The sample is other HBV genotypes, but not HBV genotype B or HBV genotype C.

For further questions or problems, please contact our technical support at trade@liferiver.com.cn