**Quantification of Hepatitis B DNA by Real-Time PCR**

**EXTRACTION OF VIRAL DNA USING**

**INSTANT VIRUS RNA/DNA KIT**

- 847-0259200602 (or 845-KS-4500050): 50 reactions
- 847-0259200603 (or 845-KS-4500250): 250 reactions

To be ordered in conjunction with RoboGene® HBV DNA Quantification Kit 2.0.

<table>
<thead>
<tr>
<th>Recommended steps before starting</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Starting material</strong></td>
<td>Serum, plasma, cell culture supernatants, Cell-free body fluids</td>
</tr>
<tr>
<td><strong>2. Lysis</strong></td>
<td>Add 400 µl Lysis Solution CLS into an Extraction Tube, Add 400 µl sample and 40 µl Proteinase K to Extraction Tube</td>
</tr>
<tr>
<td><strong>3. Binding of RNA/DNA</strong></td>
<td>Add 800 µl isopropanol, Pipetting up and down, Add Spin Filter to Receiver Tube, Add 800 µl sample to Spin Filter, 10,000 x g: 1 min, Add Spin Filter to Receiver Tube, Load residual sample, 10,000 x g: 1 min</td>
</tr>
<tr>
<td><strong>4. Washing</strong></td>
<td>Add 500 µl Washing Solution HS, 10,000 x g: 1 min, Add 650 µl Washing Solution LS, 10,000 x g: 1 min, Add 650 µl Washing Solution LS, 10,000 x g: 1 min</td>
</tr>
<tr>
<td><strong>5. Remove Ethanol</strong></td>
<td>Discard filtrate, Add Spin Filter to Receiver Tube, Centrifuge: max. speed, 5 min</td>
</tr>
<tr>
<td><strong>6. Elution</strong></td>
<td>Add Spin Filter to an Elution Tube, Add 60 µl RNAase-free water, Incubation: 2 min @ RT, 8,000 x g: 1 min</td>
</tr>
</tbody>
</table>

**Analysis**

Quantification of the eluate is performed applying the RoboGene® HBV DNA Quantification Kit 2.0

**DETECTION OF VIRAL DNA USING**

**ROBOGENE® HBV DNA QUANTIFICATION KIT 2.0**

- 847-0207500162: 136 reactions
- 847-0207500164: 72 reactions

To be ordered in conjunction with INSTANT Virus RNA/DNA Kit.

<table>
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<tr>
<td><strong>1. 5x Reagent Mix</strong></td>
<td>Add 200 µl PCR grade water, Incubation: 20 min @ 37 °C, Vortex: 3 sec, Centrifuge: shortly</td>
</tr>
<tr>
<td><strong>2. 1x Master Mix</strong></td>
<td>Add 20 µl of 1x master mix to each quantitation standard, Add 5 µl of PCR grade water, Cover the tubes</td>
</tr>
<tr>
<td><strong>3. Quantitation standards</strong></td>
<td>Add 20 µl of 1x master mix to each sample tube, Add 5 µl of eluate from RNA isolation (e.g. using the INSTANT Virus RNA/DNA) to the respective sample tubes, Cover the tubes</td>
</tr>
<tr>
<td><strong>4. Sample tubes</strong></td>
<td>HIV DNA (IU/ml) given in quantitation standards in case of using the INSTANT Virus RNA/DNA Kit</td>
</tr>
<tr>
<td><strong>5. Reaction plate</strong></td>
<td>Centrifuge: 200 x g for 1 min</td>
</tr>
<tr>
<td><strong>6. Cycling conditions CFX96</strong></td>
<td>Enzyme activation, Temperature: 95 °C, Time: 4:00 min, Repeat: 1</td>
</tr>
<tr>
<td><strong>7. Data interpretation</strong></td>
<td>HBV DNA (IU/ml) given in quantitation standards in case of using the INSTANT Virus RNA/DNA Kit</td>
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**Recommended steps before starting**

- Prepare ice-cold racks for handling of PCR reagents

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<tr>
<th>Reagent</th>
<th>Volume/Reaction</th>
<th>Final concentration</th>
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<tr>
<td>PCR grade water</td>
<td>12.1 µl</td>
<td>-</td>
</tr>
<tr>
<td>10x PCR buffer F51</td>
<td>2.5 µl</td>
<td>1 x</td>
</tr>
<tr>
<td>5x Reagent Mix</td>
<td>5 µl</td>
<td>1 x</td>
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<tr>
<td>Taq Polymerase F51</td>
<td>0.4 µl</td>
<td>2 U</td>
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**Recommended steps before starting**

- Prepare Washing Solution HS, Washing Solution LS and Proteinase K according to the instruction

**Recommended steps before starting**

- Start material: Serum, plasma, cell culture supernatants, Cell-free body fluids

**Recommended steps before starting**

- Lysis: Add 800 µl isopropanol, Pipetting up and down, Add Spin Filter to Receiver Tube, Add 800 µl sample to Spin Filter, 10,000 x g: 1 min, Add Spin Filter to Receiver Tube, Load residual sample, 10,000 x g: 1 min

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