HLA-B27 REAL-TIME PCR Genotyping Kit

Package: S (standard)

**General information**

**Intended use:** The DNA-Technology HLA-B27 REAL-TIME PCR Genotyping Kit is intended for rapid group-specific detection of HLA-B27 alleles (major histocompatibility complex, class I, B) by Real-Time PCR method in human biological samples (peripheral blood).

These alleles are generally recognized as a genetic marker of multiple disease conditions e.g. rheumatoid arthritis and ankylosing spondylitis (Bekhterev’s disease).

**Method:**
Real-time PCR qualitative analysis.

**Samples:**
Peripheral blood.

**DNA extraction:**
The DNA-Technology PREP-GS GENETICS or PREP-RAPID GENETICS kits are recommended for DNA extraction.

**Features:**
PCR-Mix contains internal control (IC B27). IC B27 serves as sample intake control and allows to evaluate the quantity of genomic DNA. It is needed for assurance of PCR quality and sufficiency of input DNA.

**Devices:**
The automatic analysis for the DNA-Technology HLA-B27 REAL-TIME PCR Genotyping Kit is available on DNA-Technology made DTlite and DPrime REAL-TIME Thermal Cyclers. Software version is 7.7.5.23 or higher; the current version of the software is available for download at [http://www.dna-technology.ru/eng/support/](http://www.dna-technology.ru/eng/support/).

⚠️ Please enquire company’s representative about compatibility of third-party Real-time instruments.

**Overall time needed to perform the analysis (not including sample preparation procedure):**
1.5 hours at average.

**The number of tests:**
48

### Content

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraffin sealed PCR-mix</td>
<td>20 μL 48 tubes or 6 8-tube strips</td>
</tr>
<tr>
<td>Taq-polymerase solution</td>
<td>500 μL 1 tube</td>
</tr>
<tr>
<td>Positive control</td>
<td>75 μL 1 tube</td>
</tr>
<tr>
<td>Mineral oil</td>
<td>1.0 mL 1 tube</td>
</tr>
<tr>
<td>Associated accessories:</td>
<td></td>
</tr>
<tr>
<td>Strip’s caps(^2)</td>
<td>6 8-caps strips</td>
</tr>
</tbody>
</table>

**Dye label detection channels corresponding to HLA-B27 alleles and IC B27**

<table>
<thead>
<tr>
<th>Fam</th>
<th>Hex</th>
<th>Rox</th>
<th>Cy5</th>
<th>Cy5.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA B27</td>
<td>IC B27</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^1\) – 4S1; 4S2; 5S1; 5S2; 6S1; 6S2 instruments.

\(^2\) – 4M1; 4M3; 4M6; 5M1; 5M3; 5M6; 6M1; 6M3; 6M6 instruments.

\(^3\) – in the case of stripped tubes use.
1. **PCR**

1.1. Mark the required number of 0.2 mL tubes with PCR-mix for each sample to be tested, for positive control (C+), for negative control (C-).

**Example:** for simultaneous testing of 4 samples in one PCR run, mark 4 tubes with PCR-mix for samples, 1 tube for "C-" and 1 tube for "C+". The resulting number of tubes is 6.

1.2. Vortex the tube with Taq-polymerase solution for 3-5 seconds and spin for 1-3 seconds to collect drops.

1.3. Add 10.0 μL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

1.4. Add one drop (~20.0 μL) of mineral oil into each tube. Close tubes tightly.

1.5. Add 5.0 μL of DNA sample into corresponding PCR-tubes. Avoid paraffin layer break. Open the tube, add DNA sample, then close the tube before proceeding to the next DNA sample to prevent contamination. Use filter tips. Do not add DNA into the "C-", "C+" tubes.

1.6. Add 5.0 μL of negative control which passed all steps of DNA extraction procedure into "C-" tubes. Add 5.0 μL of positive control into "C+" tubes. Avoid paraffin layer break.

1.7. Spin tubes briefly (1-3 sec).

1.8. Set tubes to the Thermal Cycler. It is recommended to arrange the tubes in the center of thermoblock.

1.9. Launch "RealTime PCR" application. Upload ini file “HLA_B27.ini” before the first run. Add test “HLA_B27” in subsequent runs. Specify the number and identifier of samples. Define position of the tubes in software interface according to position they were set (p. 1.8) in thermal unit. Run PCR.

2. **The PCR and post-PCR analysis** are operated by software and held in automatic mode.

3. **Interpretation of the PCR results**

The interpretation must be carried out with respect to Cp values for Fam (specific dye label) and Hex (internal control dye label) channels (see table 1).

<table>
<thead>
<tr>
<th>Fam (Fam Cp)</th>
<th>Hex (Hex Cp)</th>
<th>ΔCp = Cp (Fam) - Cp (Hex)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp is defined</td>
<td>Cp ≤32.0*</td>
<td>Less than 8.0 (positive)</td>
<td>HLA-B27 is detected</td>
</tr>
<tr>
<td>Cp is defined</td>
<td>Cp ≤32.0</td>
<td>More than 10.0 (negative)</td>
<td>HLA-B27 is not detected</td>
</tr>
<tr>
<td>Cp is not defined</td>
<td>Cp ≤32.0</td>
<td>Not considered</td>
<td></td>
</tr>
<tr>
<td>Cp is defined</td>
<td>Cp ≤32.0</td>
<td>8.0-10.0</td>
<td>Unreliable</td>
</tr>
<tr>
<td>Cp is defined/not defined</td>
<td>Cp is not defined</td>
<td>Not considered</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cp &gt;32.0</td>
<td>Not considered</td>
<td></td>
</tr>
</tbody>
</table>

In case of obtaining unreliable results, the procedure starting from the PCR amplification step must be repeated. If new test will confirm the unreliable result, the DNA extraction and PCR amplification or/and sampling procedure must be repeated (is performed sequentially).

Unreliable result can be related to the presence of PCR inhibitors in DNA sample, incorrect performance of the analysis protocol, violation of the amplification temperature regimen, etc.

**Note.** If DNA sample was obtained with PREP-RAPID GENETICS DNA Extraction Kit and ΔCp = Cp (Fam) - Cp (Hex) value falls within 8.0-10.0 range the DNA sample should be diluted 10 fold by distilled water. Take into account that Cp value by Hex channel will change. In this case the result should be considered reliable when Cp <35.0

4. **Disclaimer**

DNA-Technology Genotyping assays provide genetic information for some, but not all polymorphic loci known to be associated with certain medical conditions. This information estimates a probability of disease development but does not provide a definitive diagnosis, since other genes may contribute to the odds of disease onset. Moreover, the professional medical consultation regarding complex diseases cannot solely rely on genetic testing. The medical recommendations should also consider behavioral, physical, nutritional and familial information of a patient. On the basis of DNA-Technology Genotyping assays, a specialist can conclude whether a person of a certain genotype has lower or higher chance of disease development in relation to average risk. The definitive diagnosis is a derivative of a physicians experience and the depth of clinical information.

At the assay development stage we review the most up-to-date scientific literature on genetic associations repeatedly confirmed by independent research. We restrict our genotyping assays to a relatively small set of

* - corresponds to the 1.0 ng of genomic DNA per amplification tube.
genetic markers because we believe they provide the most helpful and unbiased information about possible genetic susceptibility to common diseases.

**Storage, transportation and handling requirements**

All components of the **HLA-B27 REAL-TIME PCR Genotyping Kit** must be stored at temperatures between 2 °C and 8 °C and out of light during the storage period. Excessive temperature and light can be detrimental to product performance.

Transportation can be held by all types of roofed transport with adherence to above mentioned temperature requirements.

Shelf life – 12 months if all the conditions of transportation, storage and operation are met.

Contact our customer service department regarding issues of quality of the **HLA-B27 REAL-TIME PCR Genotyping Kit**:

Phone: +7 (800) 200-75-15,
Phone/Fax: +7(495) 640-17-71.
E-mail: hotline@dna-technology.ru, www.dna-technology.ru.
Address: 117587, Moscow, Varshavskoye sh., 125g, building 6, DNA Technology.