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For research use only

HLA-DQB1 REAL-TIME PCR Genotyping Kit

Package: U (universal)

REF **R1-H003-N3/5EU**

General information

Intended use:
HLA-DQB1 REAL-TIME PCR Genotyping Kit is intended for simultaneous detection of 12 alleles and groups of alleles of human major histocompatibility complex DQB1 gene by polymerase chain reaction.

Method:
Real-time PCR, qualitative analysis, melting curve analysis.

Samples:
Peripheral blood.

DNA extraction:
The "DNA-Technology" PREP-GS GENETICS and PREP-RAPID GENETICS Kits are recommended for DNA extraction.

Features:
Additional round of amplification products melting - used for genotyping of some DQB1 specificities.
Simultaneous detection of several DNA-targets in one tube (multiplex).
PCR-Mix contains an internal control (DNA-IC). IC is intended for PCR quality and sufficiency of DNA assurance.
Positive control plasmid (C+) supplied with the kit is intended for specific PCR assessment.
We also recommend including in assay the negative control (C-) which is not supplied but very helpful for contamination control purposes. Use deionized water or sterile buffered saline instead of sample, starting from extraction step.

Devices:
The automatic analysis for HLA-DQB1 REAL-TIME PCR Genotyping Kit is available on "DNA-Technology" made DTlite[1], DTprime[2], and DT-96 REAL-TIME Thermal Cyclers; software version is not lower than 7.5.5.23; the current version of the software is available for download at http://www.dna-technology.ru/eng/support/.

Overall time needed to perform the analysis (excluding sample preparation procedure):
2.5 hours at average.

Number of tests:
24

Kit content

Reagent	Quantity	
• PCR-mix "DQB1-1"	480 µL	1 tube
• PCR-mix "DQB1-2"	480 µL	1 tube
• PCR-mix "DQB1-3"	480 µL	1 tube
• PCR-mix "DQB1-4"	480 µL	1 tube
• PCR-mix "DQB1-5"	480 µL	1 tube
• PCR-mix "DQB1-6"	480 µL	1 tube
• MAX Techno Taq-Polymerase	72 µL	1 tube
• PCR-buffer	1.44 mL	1 tube
• Mineral oil	2.88 mL	2 tubes
• Positive control (C+) DQ FAM	100 µL	1 tube
• Positive control (C+) DQ HEX	100 µL	1 tube

Shipping, storage and handling requirements

The PCR-mix, PCR-buffer, mineral oil, , positive control (C+) DQ FAM and positive control (C+) DQ HEX must be stored at temperatures between 2 °C and 8 °C and out of light over the storage period. The excessive temperature and light can be detrimental to product performance.
The MAX Techno Taq-Polymerase must be stored at the temperature between minus 18 °C and minus 22 °C during the storage period.
Transportation of kit's components can be held by all types of roofed transport at the temperatures corresponding to the storage conditions of individual reagents, included in the kit.
Shelf-life – 12 months from the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions.

[1] - 4S1, 4S2, 5S2, 6S1, 6S2 models
[2] - 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 models

Dye label detection channels

PCR-mix	Fam	Hex	Rox	Cy5	Cy5.5
DQB1-**1**	03	0401/0402	02	IC	-
DQB1-**2**	05	0601	0602-8	IC	-
DQB1-**3**	-	-	0302 или 0303	IC	-
DQB1-**4**	-	-	0301 или 0304	IC	-
DQB1-**5**	0305 или 0401/0402	-	-	IC	
DQB1-**6**	0501	0503	0502/0504	IC	

Procedure

1 Preparing the PCR

1.1 Mark 6 tubes for each sample and control to be tested.
Example: for simultaneous testing of 5 samples in one PCR run, mark 30 tubes for samples and 18 tubes for "C-", "C+ DQ FAM", "C+ DQ HEX". The resulting number of tubes is 48.

We recommend to include in the assay the negative control sample (C-) which has passed sample preparation procedure at least once for given batch of the reagents.

1.2 Vortex the tubes with PCR-mix for 3-5 seconds and spin for 1-3 seconds to collect drops.

1.3 Add 20 µL of PCR-mix into the marked tubes (according to the marks add to the tube 1 DQB1-1 PCR-mix, then add to the tube 2 DQB1-2 PCR-mix etc).

1.4 Vortex the tubes with PCR-buffer and MAX Techno Taq-Polymerase for 3-5 seconds and spin for 1-3 seconds to collect drops.

MAX Techno Taq-Polymerase must be stored at the temperature between minus 18 °C and minus 22 °C. The room temperature storage permitted only for a short time. Take polymerase out of refrigerator just prior to use.

1.5 Prepare the mixture of PCR-buffer and MAX Techno Taq-Polymerase. Mix in the separate tube:

- 10 x (N+1) µL of PCR-buffer;
- 0.5 × (N+1) µL of MAX Techno Taq-Polymerase,

N — number of the marked tubes including "C-", "C+ DQ FAM", "C+ DQ HEX".
Example: If you need to test 5 samples, "C-", "C+ DQ FAM" and "C+ DQ HEX" in one PCR run (48 marked tubes), mix 490 µL of PCR-buffer and 24.5 µL of MAX Techno Taq-Polymerase (calculate final volume for 49 (48+1) tubes).

1.6 Vortex the tube for 3-5 seconds and spin for 1-3 seconds to collect drops.

The mixture of PCR-buffer and MAX Techno Taq-Polymerase must be prepared just prior to use.

1.7 Add 10 µL of PCR-buffer and MAX Techno Taq-Polymerase mixture into each PCR-tube with amplification mix.

Follow the steps listed in pp 1.8 - 1.13 in the next two hours after addition of PCR-buffer and MAX Techno Taq-Polymerase mix to PCR-mix.

1.8 Add one drop (~20 µl) of mineral oil into each tube. Close tubes tightly.

The quantity of DNA should not be less then 1.0 ng per PCR reaction. The quantity of DNA should not be less then 1.0 ng per PCR reaction. The addition of less DNA amount will void the guarantee of the result reliability. The SIC REAL-TIME PCR Kit (DNA-Technology, JSC) is recommended for DNA quantity assessment.
When running both HLA DQB1 and HLA DRB1 in one PCR setup you can use the SIC result obtained for HLA DRB1. If the Cp parameter for SIC exceed 32, the reason of the uncertain and doubtful results should be attributed to insufficient quantity of DNA.

1.9 Add 5.0 µL of DNA sample into corresponding PCR-tubes (6 tubes for each sample). 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+ DQ FAM", "C+ DQ HEX" tubes.

1.10 Add 5.0 µL of "C-" which passed whole DNA extraction procedure into corresponding PCR-tubes. Add 5.0 µL of "C+ DQ FAM" into corresponding PCR-tubes. Add 5.0 µL of "C+ DQ HEX" into corresponding PCR-tubes.

1.11 Spin tubes briefly (1-3 sec).

1.12 Set the tubes to Real-time PCR instrument. Try to place tubes in the center of thermoblock.

1.13 Launch RealTime_PCR software and choose the Device handling mode. Download "HLA.ini" file if you do this test for the first time. In subsequent runs add the "DQB1" test to the protocol, specify the number and ID's of the samples, specify the position of the tubes in the thermal unit (1.8) and run PCR.

Ini.file version is «HLA_20131111.ini» or higher, «DRB1» test version is 2.1 or higher.

2 Registration and interpretation of the PCR results held in automatic mode. The HLA DQB1 gene specificities for each sample are determined by the software and taking to account total result for the given assay.

In the case of homozygous genotype repeating of PCR amplification of the same DNA preparation is recommended.

Contact our customer service department regarding issues of quality of the HLA-DQB1 REAL-TIME PCR Genotyping Kit:
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