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For research use only

HLA-DQB1 REAL-TIME PCR Genotyping Kit

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

Package: N (bulk solution)

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.
HLA-DQB1 REAL-TIME PCR Genotyping Kit can be used in scientific research practice.

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

Samples:
Peripheral blood.

DNA extraction:
The DNA-Technology's **PREP-GS Genetics** or **PREP-RAPID Genetics** extraction kits are recommended.

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 (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] REAL-TIME Thermal Cyclers; the latest version of the software is available for download at https://www.dna-technology.com/software.

Time of analysis (excluding sample preparation procedure):

from 2.5 hours.

The number of tests:

24 (including negative and positive controls in each run).

Kit contents:

Reagent	Organoleptic parameters	Quantity	
PCR-mix:	Transparent colorless liquid		
1. DQB1-1		480 µL	1 tube
2. DQB1-2		480 µL	1 tube
3. DQB1-3		480 µL	1 tube
4. DQB1-4		480 µL	1 tube
5. DQB1-5		480 µL	1 tube
6. DQB1-6		480 µL	1 tube
TechnoTaq MAX polymerase	Transparent colorless viscous liquid	72 µL	1 tube
PCR-buffer	Transparent colorless liquid	1.44 mL	1 tube
Mineral oil	Transparent colorless viscous oily liquid	2.88 mL	1 vial
Positive control (C+) DQ FAM	Transparent colorless liquid	100 µL	1 tube
Positive control (C+) DQ HEX	Transparent colorless liquid	100 µL	1 tube

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments
[2] - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments

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 or 0401/0402	-	-	IC	-
DQB1-**6**	0501	0503	0502/0504	IC	-

Procedure

1 **PCR amplification**

1. The quantity of DNA should not be less than 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, LLC) 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 32.0, the reason of the uncertain and doubtful results should be attributed to insufficient quantity of DNA.

2. The reagents and tubes should be kept away from direct sunlight!

1.1 Mark 6 tubes for each sample and control to be tested.

Example: If you need to test 5 samples in one PCR run, mark 30 tubes for samples, 6 tubes for "C-", 6 tubes for "C+ DQ FAM" and 6 tubes "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 containing PCR-mix for 3-5 s, then spin for 1-3 s to collect the 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 TechnoTaq MAX polymerase for 3-5 s, then spin for 1-3 s to collect the drops.

TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to 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 TechnoTaq MAX polymerase. Add into the separate tube:

- 10×(N+1) µL of PCR-buffer;
- 0.5×(N+1) µL of TechnoTaq MAX polymerase;

N — number of the marked tubes including "C-","C+ DQ FAM", "C+ DQ HEX".

Example: For simultaneous testing of 5 samples, "C+ DQ FAM" and "C+ DQ HEX" in one PCR run (48 tubes), mix 490 µL of PCR-buffer and 24.5 µL of TechnoTaq MAX polymerase (calculate final volume for 49 (48+1) tubes).

1.6 Vortex the tube for 3-5 s, then spin for 1-3 s to collect the drops.

The mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared just prior to use.

1.7 Add 10 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each PCR-tube.

Follow the steps listed in pp 1.8 - 1.14 within two hours after addition of PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

1.8 Add one drop (~20 µL) of mineral oil in each PCR-tube. Close tubes.

1.9 Vortex the tubes with samples, "C-", "C+ DQ FAM" and "C+ DQ HEX" for 3-5 s and spin down the drops on vortex mixer for 1-3 s.

1. In case of using **PREP-GS Genetics DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g)16000 for one minute at room temperature (from 18 °C to 25 °C) to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 s in a vortex mixer.

2. Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next tube to prevent contamination. Use filter tips. Close tubes tightly.

1.10 Add 5.0 µL of DNA sample into corresponding PCR-tubes (6 tubes for each sample). Do not add DNA into the "C-", "C+ DQ FAM", "C+ DQ HEX" tubes.

1.11 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.

1.12 Spin the tubes for 1–3 s to collect the drops.

1.13 Set the tubes to real-time PCR thermal cycler.

1.14 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID's of the samples and negative control samples. Specify the position of the tubes in the thermal unit (see 1.13) and run PCR.

2 **Data collection and data analysis.**

Registration and interpretation of the PCR results are 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.

Storage, shipping and handling requirements

All components of the **HLA-DQB1 REAL-TIME PCR Genotyping Kit**, except the TechnoTaq MAX polymerase, must be stored at temperatures from 2 °C to 8 °C during the storage period. PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

Excessive temperature and light can be detrimental to product performance.

The kit must to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

Transportation of the kit, except the TechnoTaq MAX polymerase, is allowed in thermoboxes with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the TechnoTaq MAX polymerase in thermoboxes with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

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

Contact our customer service department regarding quality issues with the kit:

8 800 200-75-15 (toll-free call for Russia)

+7 (495) 640-16-93 (chargeable call for CIS and foreign countries)

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Key to symbols

	Temperature limit		Consult instructions for use		Catalogue number
	Use-by date		Manufacturer		Batch code
	Date of manufacture		Contains sufficient for <n> tests		Keep away from sunlight
	Caution		Non-sterile		

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[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[2] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.