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For research use only

HLA-DRB1 REAL-TIME PCR Genotyping Kit

REF **R1-H001-S3/5EU**

Package: S

General information

Intended use:
HLA-DRB1 REAL-TIME PCR Genotyping Kit is intended for simultaneous detection of 13 alleles and groups of alleles of human major histocompatibility complex DRB1 gene by polymerase chain reaction.
HLA-DRB1 REAL-TIME PCR Genotyping Kit can be used in scientific research practice.

Method:
Real-time PCR, qualitative analysis.

Samples:
Peripheral blood.

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

Features:
Simultaneous detection of several DNA-targets in one tube (multiplex).
The DRB1 internal control (IC DRB1) in the tubes 1, 3-8 of the strip A and tubes 1-7 of the strip B aids the assessment of the results in each PCR tube.
Internal control (IC) in the tube 2 of the strip A is needed for DNA addition assessment.
Internal control (IC) in the tube 8 of the strip B is needed for PCR quality assessment.
Assay includes Sample Intake Control (SIC), which is intended for extraction quality assessment as well as for evaluation of sufficiency of sample for obtaining reliable result.
Assay includes Marker, which is intended to control the correct position of a strip in thermal unit.
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-DRB1 REAL-TIME PCR Genotyping Kit** is available on "DNA-Technology" made DTlite[1] and 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 one positive control and one negative control in each run).

Kit contents:

Reagent	Organoleptic parameters	Quantity	
1. Paraffin sealed PCR-mix:			
Strip A	Transparent colorless or blue liquid under white wax layer	20 µL in each	24 8-tubes strips
Strip B	Transparent colorless or blue liquid under blue wax layer	20 µL in each	24 8-tubes strips
2. Taq-polymerase solution	Transparent colorless liquid	1.0 mL	4 tubes
3. Mineral oil	Transparent colorless viscous oily liquid	8.0 mL	1 vial
4. Positive control(C+) DRB1	Transparent colorless liquid	160 µL	2 tubes
Associated accessories:			
Strip's caps			48 8-caps

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

Strip content, colour codes and detection channels

№ of tube in strip	Detection channels					PCR-mix color	Paraffin color
	Fam	**Hex**	**Rox**	**Cy5**	**Cy5.5**		
Strip A							
1	01	IC DRB1	-	-	-	Blue	White
2	03	IC	-	-	-	Colorless	
3	04	IC DRB1	-	-	-		
4	08		Marker	-	-		
5	09		-	-	-		
6	11		-	-	-		
7	12		-	-	-		
8	13a		-	-	-		
Strip B							
1	13b	IC DRB1	-	-	-	Blue	Blue
2	14-1		-	-	-	Colorless	
3	14-2		-	-	-		
4	15		-	-	-		
5	16		-	-	-		
6	07		-	-	-		
7	10		-	-	-		
8	SIC	IC	Marker	-	-		

Procedure

1 PCR amplification

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

Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

1.1 Mark one strip A and one strip B for each sample and control to be tested.

Example: for simultaneous testing of 4 samples in one PCR run, mark 4 strips A and 4 strips B for samples, 1 strip A and 1 strip B for "C-"; 1 strip A and 1 strip B for "C+". The resulting number of strips is 12.

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

1.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

1.4 Add one drop (~20 µL) of mineral oil into each tube of the strip. Close strips.

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 strip, add DNA sample (or control sample), then close the strip before proceeding to the next strip to prevent contamination. Use filter tips. Close strips tightly.

1.5 Add 5.0 µL of DNA sample into corresponding PCR-tubes. Avoid paraffin layer break. Use filter tips. Do not add DNA into the "C-", "C+" tubes.

1.6 Add 5.0 µL of negative control sample (C-) which passed whole DNA extraction procedure into "C-" strips. Add 5.0 µL of positive control sample (C+) into "C+" strips.

1.7 Spin strips briefly (1-3 sec).

1.8 Set the strips into the thermal cycler.

1.9 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and IDs of the samples, positive and negative control samples. Specify the position of the strips in the thermal unit (see 1.8) and run PCR.

2 Data collection and data analysis.

Registration of the PCR results is held in automatic mode.
The HLA DRB1 gene specificities for each sample is determined by the software and taking to account total result for the given assay. At that the sample under study can be characterized either: by one specificity and by combination of specificities (see Annex, Table 1).
In the case of homozygous genotype repeating of analysis for the same DNA preparation is recommended.

The quantity of DNA to be analyzed must be greater than or equal to 1.0 ng per reaction (the Cp parameter for SIC must not be more than 32.0). 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.

[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.

If the sample characterized by the second tube of the strip A (*03) and the third tube of the strip B (*14-2) and at the same time Cp parameter is ≤29.0, the genotyping result for the given sample will be considered doubtful. In this case dilute the sample 10-fold and repeat the assay for the given sample. You can use the elution buffer from the DNA extraction kit for this purpose (when using **PREP-RAPID Genetics DNA Extraction Kit** you can use the "C-" which has passed the sample preparation procedure).

Storage, shipping and handling requirements

All components of the **HLA-DRB1 REAL-TIME PCR Genotyping Kit** 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.

Excessive temperature and light can be detrimental to product performance.

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

Transportation of the kit is allowed in thermobox 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.

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)

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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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		

Table 1. Interpretation of the PCR results for genotyping DRB1 gene

№ of strip	№ of tube	Mix	Specificity of HLA DRB1 gene														
			*01	*03	*04	*07	*08	*09	*10	*11	*11	*12	*13	*14	*14	*15	*16
A	1	01	+														
	2	03		+													
	3	04			+												
	4	08					+										
	5	09						+									
	6	11								+	+						
	7	12										+					
	8	13a		+									+	+/-	+		
B	1	13b								+/-			+		+		
	2	14-1									+			+			
	3	14-2		?[1]											+[2]		
	4	15														+	
	5	16															+
	6	07				+											
	7	10							+								

Legend:
+ - always works;
+/- - can work or not.

[1] - If the sample characterized by the second tube of the strip A (*03) and the third tube of the strip B (*14-2) and at the same time Cp parameter is ≤29, the genotyping result for the given sample will be considered doubtful. In this case dilute the sample 10-fold and repeat the assay for the given sample.
[2] - If the sample is characterized by the tubes 13-a, 13-b, 14-2, the genotype of the sample is defined as *13/14,*14 (i.e. *13,*14 or *14,*14)