DNA-TECHNOLOGY

For research use only

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Phenylketonuria Screen REAL-TIME PCR Genotyping Kit

REF **R1-H950-N3/4EU**

Package: N (bulk solution)

General information

Intended use:

Phenylketonuria Screen REAL-TIME PCR Genotyping Kit is intended for detection of the 4 most common genetic polymorphisms associated with inherited risk of phenylketonuria.

Method:

Real-time PCR followed by melting curve analysis, qualitative analysis.

Samples:

Peripheral blood.

DNA extraction:

The "DNA-Technology" PREP-GS Genetics kit is recommended for DNA extraction.

Features:

Two alleles are detected simultaneously in single tube.

PCR-Mix contains an internal control (DNA-IC). IC is intended for PCR quality assurance.

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 Phenylketonuria Screen 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 http://www.dna-technology.com/software.

Overall time needed to perform the analysis (excluding sample preparation procedure):

from 2 hours.

The number of tests:

48[3]

Kit contents:

Reagent	Quantity	
• PCR-mix:		
1. PAH: R261Q	960 µL	1 tube
2. PAH: R408W	960 µL	1 tube
3. PAH: IVS10nt546	960 µL	1 tube
4. PAH: IVS12+1G>A	960 µL	1 tube
• PCR-buffer	960 µL	2 tubes
• Taq-AT-polymerase	96 µL	1 tube
• Mineral oil	3,84 mL	1 vial

Dye label detection channels corresponding to allelic variants and IC

PCR-mix	Fam	Hex	Rox	Cy5	Cy5.5
All PAH mixes	N (norm)	m (mutation)	-	IC	-

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments.
[2] - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments.
[3] - 46 analyzed samples and 2 negative control samples at two DTprime (*M*) runs with full load of device thermoblock.

Procedure

1 PCR amplification

⚠ The quantity of DNA to be analyzed must be greater than or equal to 1.0 ng per reaction (the Cp parameter for IC must not be more than 32.0). The violation of this requirement will affect the validity of analysis and void the manufacturer guarantee.

1.1 Mark the required number of 0.2 mL PCR-tubes for each polymorphism to be tested (one tube for each sample and one for negative control "C-")

Example. If you need to test 5 samples, mark 6 tubes of each PCR-mix: 5 for the samples and 1 for the "C-". Total number of tubes – 24.

	PAH: R261Q	PAH: R408W	PAH: IVS10nt546	PAH: IVS12+1G>A
Sample 1	√	√	√	√
Sample 2	√	√	√	√
Sample 3	√	√	√	√
Sample 4	√	√	√	√
Sample 5	√	√	√	√
«C–»	√	√	√	√

1.2 Vortex the tubes for 3-5 seconds, then spin for 1-3 seconds to collect the drops.
1.3 Add 20 µL of corresponding PCR-mix into the marked tubes (use a new pipette tip for each type of PCR-mix).
1.4 Vortex the tubes with PCR-buffer and Taq-AT-polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

⚠ Taq-AT-polymerase must be stored at minus 20 °C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

1.5 Prepare the mixture of PCR-buffer and Taq-AT-polymerase. Mix in the separate tube:
- 10×(N+1) µL of PCR-buffer,
- 0.5×(N+1) µL of Taq-AT-polymerase,

N — number of the marked tubes including "C-".

Example: For simultaneous testing of 5 samples and 1 "C-" (resulting number of marked tubes is 24), prepare mix of PCR-buffer and Taq-AT-polymerase for 25 tubes, i.e. mix 250 µL of PCR-buffer with 12.5 µL of Taq-AT-polymerase.

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

⚠ The mixture of PCR-buffer and Taq-AT-polymerase must be prepared just prior to use.

1.7 Add 10 µL mixture of PCR-buffer and Taq-AT-polymerase into each PCR-tube.

⚠ Follow the steps listed in pp 1.8 - 1.13 within two hours after addition of PCR-buffer and Taq-AT-polymerase mix to amplification mix.

1.8 Add one drop (~20 µL) of mineral oil in each PCR-tube. Close the tubes tightly.
1.9 Add 5.0 µL of the DNA sample into each tube assigned to test samples (4 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-" tubes.
1.10 Add 5.0 µL of negative control ("C-"), which passed all steps of DNA extraction procedure into corresponding tubes.
1.11 Spin the tubes for 1–3 seconds to collect the drops.
1.12 Set the tubes to Real-time PCR instrument.
1.13 Launch the RealTime_PCR application in Device handling mode. Upload Phenylketonuria_Screen_en.ini file before the first run. In subsequent runs add tests "Phenylketonuria_Screen" (or use multitest mode). Specify the number and identificator of samples. Define position of tubes in software interface according to position they were set in the thermoblock (see 1.12). Run PCR.

⚠ The type of the negative control tubes must be specified as "Sample".

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

For samples containing a sufficient quantity of DNA for correct analysis, the software defines the genotype. The samples containing an insufficient quantity of DNA (less than 1.0 ng per reaction or Cp Cy5>32.0) will be analyzed as N/A (uncertain result).

⚠ It is recommended to repeat genotyping of homo-and heterozygous mutant samples, starting from the DNA extraction step.

Storage and handling requirements

The PCR-mix, PCR-buffer, mineral oil must be stored between 2 °C and 8 °C and out of light during the storage period. Excessive temperature and light can be detrimental to product performance.
The Taq-AT-polymerase must be stored at temperatures between minus 18 °C and minus 22 °C during the storage period.

Note:

1. Tubes with PCR-mix, PCR buffer and mineral oil can be transported at the temperature between 0 °C and 24 °C for not more than 72 hours.
2. Tube with Taq-AT-polymerase can be transported on ice (in a box with freezable gel-type packets) for not more than 72 hours.

Shelf life – 12 months in compliance with all transportation, storage and operation conditions.

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

Table 1.Genotypes and melting temperatures (only for DTlite, DTprime instruments)

Polymorphism	Homozygote Fam/Fam			Homozygote Hex/Hex			Heterozygote		
	Genotype	Fam,°C	Hex,°C	Genotype	Fam,°C	Hex,°C	Genotype	Fam,°C	Hex,°C
PAH: R261Q	**NN**	55,0	47,8	**mm**	42,5	53,2	**Nm**	55,0	52,5
PAH: R408W	**NN**	54,2	44,3	**mm**	46,0	55,0	**Nm**	53,0	54,0
PAH: IVS10nt546	**NN**	51,9	39,0	**mm**	41,7	49,9	**Nm**	50,8	49,3
PAH: IVS12+1G>A	**NN**	49,0	38,9	**mm**	41,7	48,0	**Nm**	49,0	47,9

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