DNA-TECHNOLOGY

For research use only

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FAMILIAL MEDITERRANEAN FEVER
REAL-TIME PCR Genotyping Kit

REF R1-H952-N3/4EU

Package: N (bulk solution)

General information

Intended use:
FAMILIAL MEDITERRANEAN FEVER REAL-TIME PCR Genotyping Kit is intended for the identification of MEFV gene mutations.

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

Samples:
Peripheral blood.

DNA extraction:
The "DNA-Technology" PREP-GS Genetics or PREP-MP Genetics kits are 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 DNA sufficiency control and assurance of PCR quality.
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 **FAMILIAL MEDITERRANEAN FEVER 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):
From 2 hours.

The number of tests:
48

Dye label detection channels corresponding to allelic variants and IC

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

Shipping and storage requirements

The PCR-mix, PCR-buffer and mineral oil 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 Taq-AT-polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.
Shelf life of **FAMILIAL MEDITERRANEAN FEVER REAL-TIME PCR Genotyping Kit** – 12 months if all the conditions of transportation, storage and operation are met.

Contact our customer service department regarding quality issues with the **FAMILIAL MEDITERRANEAN FEVER REAL-TIME PCR Genotyping Kit**:
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[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments
[2] - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments

Kit contents:

Reagent	Quantity	
• PCR-Mix:		
1. MEFV: 1437 C>G (F479L)	960 µL	1 tube
2. MEFV: 2040 G>C (M680I (G/C))	960 µL	1 tube
3. MEFV: 2076_2078del (I692del)	960 µL	1 tube
4. MEFV: 2040 G>A (M680I (G/A))	960 µL	1 tube
5. MEFV: 2080 A>G (M694V)	960 µL	1 tube
6. MEFV: 2082 G>A (M694I)	960 µL	1 tube
7. MEFV: 2177 T>C (V726A)	960 µL	1 tube
8. MEFV: 2084 A>G (K695R)	960 µL	1 tube
9. MEFV: 2230 G>T (A744S)	960 µL	1 tube
10. MEFV: 2282 G>A (R761H)	960 µL	1 tube
11. MEFV: 1105 C>T (P369S)	960 µL	1 tube
12. MEFV: 1223 G>A (R408Q)	960 µL	1 tube
• PCR-Buffer	1.0 mL	6 tubes
• Taq-AT-polymerase	300 µL	1 tube
• Mineral oil	12 mL	1 vial

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 (12 tubes for each sample and 12 for negative control "C-")

Example. If you need to test 5 samples, mark 12 tubes of each PCR-mix: 60 for the samples and 12 for the "C-". Total number of tubes – 72.

1.2 Vortex the tubes with PCR-mixes 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 72), prepare mix of PCR-buffer and Taq-AT-polymerase for 73 tubes, i.e. mix 730 µL of PCR-buffer with 36.5 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 of PCR-buffer and Taq-AT-polymerase mixture into each PCR-tube.

⚠ Follow the steps listed in pp 1.9 - 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.

1.9 Add 5.0 µL of the DNA sample into each tube assigned to test samples (12 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.

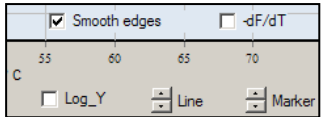
1.11 Spin the tubes for 1–3 seconds to collect the drops.

1.12 Set the tubes to Real-time PCR instrument.

1.13 Launch the RealTime_PCR application in Device operation mode. Upload MEFV.ini file before the first run. In subsequent runs add tests "MEFV" (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** is operated by software and held in automatic mode. It is recommended to use the "Smooth edges" function when analyzing melting curves.



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

⚠ In case when for the sample heterozygote (N/mut) or homozygote for the mutation (mut/mut) for one of the polymorphisms in the group [2076_2078del, 2080 A>G, 2082 G>A, 2084 A>G] is identified,, other polymorphisms in this group are allowed to be "N/A" or "?" and interpreted as normal homozygote (N/N). If for both of the two polymorphisms in the specified group the result of genotyping is "mutant homozygote" (mut/mut), the result must be interpreted as the compound heterozygotes for these polymorphisms.

In case when for the sample heterozygote (N/mut) or homozygote for the mutation (mut/mut) for one of the polymorphisms in the group [2040 G>A, 2040 G>C] is identified, other polymorphism in this group is allowed to be "N/A" or "?" and interpreted as normal homozygote (N/N). If for both of the two polymorphisms in the specified group result of genotyping is "mutant homozygote" (mut/mut), the result must be interpreted as the compound heterozygotes for these polymorphisms.

⚠ We recommend to retest heterozygous and mutant samples, starting from the DNA extraction step.

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