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For research use only

Cystic Fibrosis – rare CFTR mutations
REAL-TIME PCR Genotyping Kit

REF **R1-H948-N3/4EU**

Package: N (bulk solution)

General information

Intended use:
Cystic Fibrosis – rare CFTR mutations REAL-TIME PCR Genotyping Kit is intended for detection of the 16 relatively rare genetic polymorphisms associated with inherited risk of cystic fibrosis.
Cystic Fibrosis – rare CFTR mutations REAL-TIME PCR Genotyping Ki can be used in scientific research practice.

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

Samples:
Peripheral blood.

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

Features:
Two alleles are detected simultaneously in single tube.
In PCR-mix for each polymorphism the system for human genomic DNA amplification (IC) is included. It allows to control quantity of human DNA in amplification tube to exclude mistakes in genotyping.
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 **Cystic Fibrosis – rare CFTR mutations 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 hours.

The number of tests:

48 (including negative controls in each run).

Dye label detection channels corresponding to allelic variants and IC

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

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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	Organoleptic parameters	Quantity	
PCR-mix:	Transparent colorless liquid		
1. CFTR: L138ins		960 µL	1 tube
2. CFTR: G542X		960 µL	1 tube
3. CFTR: R117H		960 µL	1 tube
4. CFTR: 604insA		960 µL	1 tube
5. CFTR: 621+1G>T		960 µL	1 tube
6. CFTR: S1196X		960 µL	1 tube
7. CFTR: 3821delT		960 µL	1 tube
8. CFTR: 3667insTCAA		960 µL	1 tube
9. CFTR: R334W		960 µL	1 tube
10. CFTR: 394delTT		960 µL	1 tube
11. CFTR: R553X		960 µL	1 tube
12. CFTR: K598ins		960 µL	1 tube
13. CFTR: 2184insA		960 µL	1 tube
14. CFTR: 2183AA>G		960 µL	1 tube
15. CFTR: 2789+5G>A		960 µL	1 tube
16. CFTR: 3944delGT		960 µL	1 tube
PCR-buffer	Transparent colorless liquid	3.84 mL	2 vials
Taq-AT-polymerase	Transparent colorless viscous liquid	192 µL	2 tubes
Mineral oil	Transparent colorless viscous oily liquid	7.68 mL	2 vials

Procedure

1 PCR amplification

1. 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.
2. The reagents and tubes should be kept away from direct sunlight!

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 – 96.

PCR tubes marking

	CFTR: L138ins	CFTR: G542X	CFTR: R117H	CFTR: 604insA	CFTR: 621+1G>T	CFTR: S1196X	CFTR: 3821delT	CFTR: 3667insTCAA	CFTR: R334W	CFTR: 394delTT	CFTR: R553X	CFTR: K598ins	CFTR: 2184insA	CFTR: 2183AA>G	CFTR: 2789+5G>A	CFTR: 3944delGT
Sample 1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Sample 2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Sample 3	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Sample 4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Sample 5	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
"C-"	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

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 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 s, then spin for 1-3 s to collect the drops.

Taq-AT-polymerase must be stored at temperatures from minus 18°C to minus 22°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 96) in one PCR run, mix 970 µL of PCR-buffer and 48.5 µL of Taq-AT-polymerase (calculate final volume for 97 (96+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 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 1.8 - 1.14 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 Vortex the tubes with samples and "C-" for 3-5 s and spin down the drops by centrifuging 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, 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 the DNA sample into each tube assigned to test samples (16 tubes for each sample).Do not add DNA into the "C-" tubes.

1.11 Add 5.0 µL of negative control (C-), which passed all steps of DNA extraction procedure into corresponding 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 instrument1. Add corresponding test2, 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.

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

2 Data collection and data analysis.

Registration and interpretation of the PCR results are 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.

[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.

Storage, shipping and handling requirements

All components of the **Cystic Fibrosis – rare CFTR mutations REAL-TIME PCR Genotyping Kit**, except the Taq-AT-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 Taq-AT-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 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, except the Taq-AT-polymerase, is allowed in termobox 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 Taq-AT-polymerase in termobox 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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