



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

**Package: U (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.

**Method:**

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

**Samples:**

Peripheral blood.

**DNA extraction:**

The “DNA-Technology” PREP-GS Genetics or PREP-RAPID 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 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 Cystic Fibrosis – rare CFTR mutations REAL-TIME PCR Genotyping Kit is available on “DNA-Technology” made DTlite<sup>1</sup>, DTprime<sup>2</sup> and DT-96 REAL-TIME Thermal Cyclers; software version is not lower than 7.3.5.84; the current version of the software is available for download at <http://www.dna-technology.ru/eng/support/>.



Please enquire DNA-Technology company's representative about compatibility of third-party Real-time instruments.

**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 CFTR mixes	N (norm)	m (mutation)	-	IC	-

<sup>1</sup> - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments

<sup>2</sup> - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments

## Kit contents:

Reagent	Quantity	
• PCR-Mix:		
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: 3821delIT	960 µL	1 tube
8. CFTR: 3667insTCAA	960 µL	1 tube
9. CFTR: R334W	960 µL	1 tube
10. CFTR: 394delIT	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	3.84 mL	2 vials
• Taq-AT-polymerase	192 µL	2 tubes
• Mineral oil	7.68 mL	2 vials

## 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 – 96.

#### PCR tubes marking

	CFTR: L138ins	CFTR: G542X	CFTR: R117H	CFTR: 604insA	CFTR: 621+1G>T	CFTR: S1196X	CFTR: 3821delIT	CFTR: 3667insTCAA	CFTR: R334W	CFTR: 394delIT	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 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 96), prepare mix of PCR-buffer and Taq-AT-polymerase for 97 tubes, i.e. mix 970 µL of PCR-buffer with 48.5 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 tightly.

**1.9** Add 5.0 µL of the DNA sample into each tube assigned to test samples (16 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 CFTR\_rare\_en.ini file before the first run. In subsequent runs add tests "CFTR" (or use multitest mode). Specify the number and identifier 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 and 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.

Shelf life of Cystic Fibrosis – rare CFTR mutations REAL-TIME PCR Genotyping Kit – 12 months since the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions.

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](mailto:hotline@dna-technology.ru), [www.dna-technology.ru](http://www.dna-technology.ru).

Address: "DNA-Technology" LLC, 117587, Russia, Moscow, int. ter. Municipal District Chertanovo Severnoye, Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12.

**Appendix.**

Table 1

Genotypes and melting temperatures (only for DTlite, DTprime or DT-96 instruments)

Polymorphism	Homozygous Fam/Fam			Homozygous Hex/Hex			Heterozygous		
	Genotype	Fam, °C	Hex, °C	Genotype	Fam, °C	Hex, °C	Genotype	Fam, °C	Hex, °C
CFTR: L138ins	<b>NN</b>	56.1	39.6	<b>mm</b>	43.2	56.9	<b>Nm</b>	55.4	56.3
CFTR: G542X	<b>NN</b>	52.0	46.6	<b>mm</b>	42.1	51.6	<b>Nm</b>	52.0	51.6
CFTR: R117H	<b>NN</b>	53.4	44.5	<b>mm</b>	44.1	54.0	<b>Nm</b>	53.1	54.0
CFTR: 604insA	<b>NN</b>	52.1	45.6	<b>mm</b>	46.1	52.3	<b>Nm</b>	51.5	51.6
CFTR: 621+1G>T	<b>NN</b>	49.4	41.2	<b>mm</b>	44.1	49.3	<b>Nm</b>	48.9	48.7
CFTR: S1196X	<b>NN</b>	50.8	38.6	<b>mm</b>	41.2	51.7	<b>Nm</b>	50.2	51.3
CFTR: 3821delT	<b>NN</b>	50.6	44.9	<b>mm</b>	42.2	50.9	<b>Nm</b>	50.3	50.5
CFTR: 3667insTCAA	<b>NN</b>	54.8	47.9	<b>mm</b>	47.6	54.1	<b>Nm</b>	54.3	53.6
CFTR: R334W	<b>NN</b>	44.2	32.6	<b>mm</b>	36.6	41.8	<b>Nm</b>	43.7	41.3
CFTR: 394delTT	<b>NN</b>	50.2	46.2	<b>mm</b>	39.4	52.3	<b>Nm</b>	49.6	51.8
CFTR: R553X	<b>NN</b>	58.8	51.9	<b>mm</b>	51.7	60.5	<b>Nm</b>	58.4	59.9
CFTR: K598ins	<b>NN</b>	52.5	47.1	<b>mm</b>	44.0	53.7	<b>Nm</b>	52.0	53.3
CFTR: 2184insA	<b>NN</b>	49.8	44.5	<b>mm</b>	45.2	50.1	<b>Nm</b>	49.5	50.1
CFTR: 2183AA>G	<b>NN</b>	49.8	37.5	<b>mm</b>	39.5	50.3	<b>Nm</b>	49.4	49.9
CFTR: 2789+5G>A	<b>NN</b>	56.9	48.1	<b>mm</b>	50.4	55.2	<b>Nm</b>	56.7	55.0
CFTR: 3944delGT	<b>NN</b>	55.8	46.8	<b>mm</b>	41.0	55.9	<b>Nm</b>	55.3	55.4