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For research use only

CHEK2 mutations Real-Time PCR Genotyping Kit

REF **R1-H967-N3/4EU**

Package: N (bulk solution)

CHEK2 mutations Controls Kit[1]

REF **C-020EU**

General information

Intended use:
CHEK2 mutations Real-time PCR Genotyping Kit and **CHEK2 mutations Controls Kit** are intended for detection and allelic discrimination of human CHEK2 gene polymorphisms (mutations 1100delC, IVS2+1G>A, 470T>C (Ile157Thr)) by method of Real-Time PCR.
CHEK2 mutations Real-time PCR Genotyping Kit and **CHEK2 mutations Controls Kit** 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:
Several 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 the negative control (C-) in an assay which is not supplied but is 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 **CHEK2 mutations Real-time PCR Genotyping Kit** is available on "DNA-Technology" made DTlite[2], DTprime[3] 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 and positive controls in each run).

Dye label detection channels corresponding to allelic variants and IC

PCR-mix	Polymorphism (mutation)	Fam	Hex	Rox	Cy5	Cy5.5
CHEK2:1100delC	CHEK2:1100delC	Ins	Del	-	IC	-
CHEK2:IVS2+1G>A	CHEK2:IVS2+1G>A	G	A			
CHEK2:470T>C (Ile157Thr)	CHEK2:470T>C (Ile157Thr)	T	C			

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Reason: MoldSign Signature
Location: Moldova

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

Kit contents:

Reagent	Organoleptic parameters	Quantity	
CHEK2 mutations Real-time PCR Genotyping Kit			
PCR-mix:	Colorless transparent liquid		
1. CHEK2:1100delC		960 µL	1 tube
2. CHEK2:IVS2+1G>A		960 µL	1 tube
3. CHEK2:470T>C (Ile157Thr)		960 µL	1 tube
PCR-buffer	Colorless transparent liquid	1.0 mL in each	2 tubes
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	72 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL in each	3 tubes
Positive control[4] [homozygous for normal allele for all gene polymorphisms][5]	Colorless transparent liquid	130 µL	1 tube
CHEK2 mutations Controls Kit			
Positive controls:			
1. C+1 [CHEK2:1100delC allele heterozygous]		50 µL	1 tube
2. C+2 [CHEK2:IVS2+1G>A allele heterozygous]	Colorless transparent liquid	50 µL	1 tube
3. C+3 [CHEK2:470T>C (Ile157Thr) allele heterozygous]		50 µL	1 tube
4. C+4 [CHEK2:470T>C (Ile157Thr) mutant allele homozygous]		50 µL	1 tube

Genotypes, determined in positive control samples

PCR-mix	Polymorphism (mutation)	Genotype				
		C+	Positive control			
			C+1	C+2	C+3	C+4
CHEK2:1100delC	CHEK2:1100delC	Ins/Ins	Ins/Del	Ins/Ins	–	–
CHEK2:IVS2+1G>A	CHEK2:IVS2+1G>A	G/G	G/G	G/A	–	–
CHEK2:470T>C (Ile157Thr)	CHEK2:470T>C (Ile157Thr)	T/T	–	–	T/C	C/C

Procedure

1 PCR amplification

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

2. 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, negative control "C-" and positive control "C+").

Example. If you need to test 6 samples, mark 8 tubes of each PCR-mix: six for the samples, one for the "C-" and one for the "C+". Total number of tubes – 24.

PCR tubes marking

	CHEK2:1100delC	CHEK2:IVS2+1G>A	CHEK2:470T>C(Ile157Th
Sample 1	√	√	√
Sample 2	√	√	√
Sample 3	√	√	√
Sample 4	√	√	√
Sample 5	√	√	√
Sample 6	√	√	√
"C–"	√	√	√
"C+"	√	√	√

[4] - marking as C+ (homozygous for normal allele for all gene polymorphisms) is allowed
[5] - is intended for 10 tests

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 TechnoTaq MAX polymerase for 3-5 s, then spin for 1-3 s to collect the drops.

TechnoTaq MAX 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 TechnoTaq MAX polymerase. Add into one tube:

- 10×(N+1) µL of PCR-buffer;
- 0.5×(N+1) µL of TechnoTaq MAX polymerase;

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

Example: For simultaneous testing of 6 samples, 1 "C-" and 1 "C+" (resulting number of marked tubes is 24), prepare mix of PCR-buffer and TechnoTaq MAX polymerase for 25 (24+1) tubes, i.e. mix 250 µL of PCR-buffer with 12.5 µL of TechnoTaq MAX polymerase.

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 TechnoTaq MAX polymerase must be prepared just prior to use.

1.7 Add 10 µL of PCR-buffer and TechnoTaq MAX 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 TechnoTaq MAX 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, "C-" and "C+" for 3-5 s and spin down the drops 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.

Relative centrifugal force (RCF or g) depends on rotation frequency and rotor radius (Annex A). To establish if your centrifuge meets the requirements apply to the exploitation manual for centrifuge.

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 (3 tubes for each sample). Do not add DNA into the "C-", "C+" tubes.

1.11 Add 5.0 µL of negative control (C-) which passed all steps of DNA extraction procedure into corresponding tubes. Add 5.0 µL of positive control (C+) 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 instrument[6]. Add corresponding test[7], 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 and positive controls 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>32.0) will be analyzed as N/A (uncertain result).

Because of the high medical and social significance of CHEK2 mutation carrier status, we recommend to retest mutant allele homo- and heterozygous samples, starting from the DNA extraction step.
During the retesting procedure appropriate control sample from **CHEK2 mutations Controls Kit** should be used instead of "C+" from CHEK2 mutations Real-time PCR Genotyping Kit.

[6] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[7] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Scheme of CHEK2 positive controls application for repeated genotyping

PCR-mix	Polymorphism (mutation)	Genotype	Control sample			
			C+1	C+2	C+3	C+4
CHEK2:1100delC	CHEK2:1100delC	Ins/Del	+	–	–	–
CHEK2:IVS2+1G>A	CHEK2:IVS2+1G>A	G/A	–	+	–	–
CHEK2:470T>C (Ile157Thr)	CHEK2:470T>C (Ile157Thr)	T/C	–	–	+	–
		C/C	–	–	–	+

Storage, shipping and handling requirements

The PCR-mix, PCR-buffer, mineral oil and positive controls 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 TechnoTaq MAX 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.

It is allowed to transport kits in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions.

Transportation of the **CHEK2 mutations Real-time PCR Genotyping Kit**, except the TechnoTaq MAX polymerase and **CHEK2 mutations Controls 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. Kits should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the TechnoTaq MAX polymerase in thermobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days. TechnoTaq MAX polymerase 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)

E-mail: hotline@dna-technology.ru, https://www.dna-technology.com

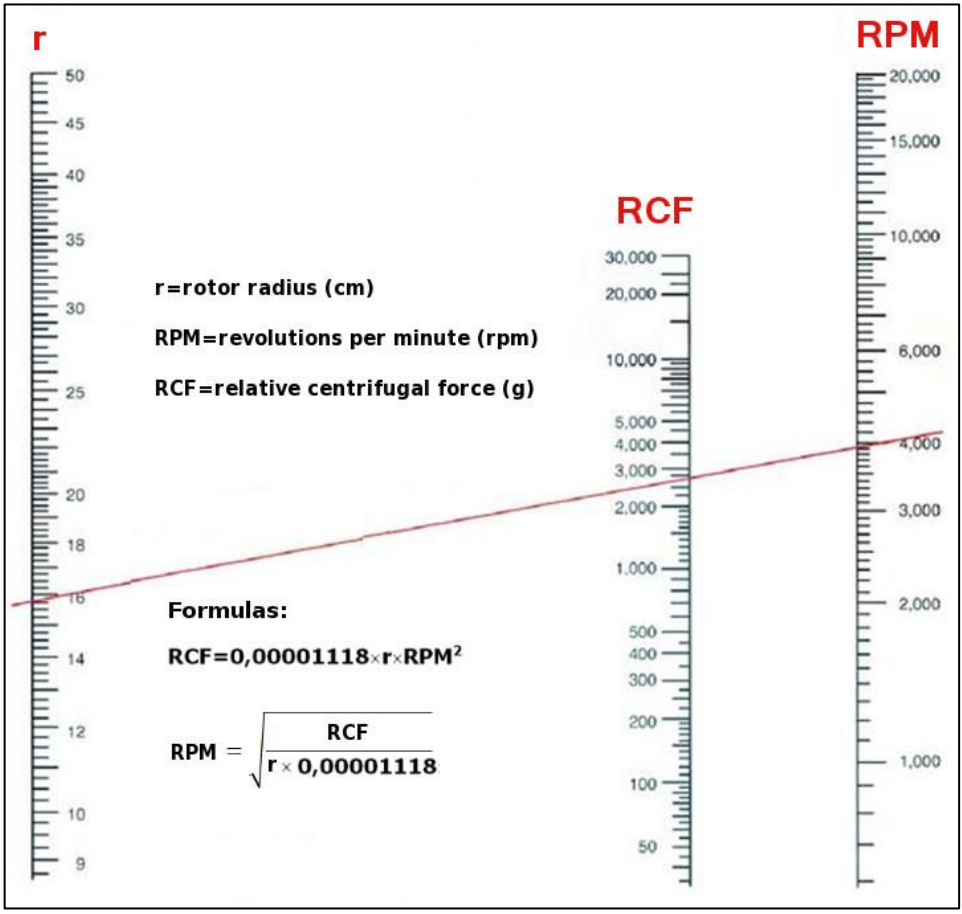
Address: "DNA-Technology" LLC, 117585, Russia, Moscow, int. ter. Municipal District Chertanovo Severnoye, Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12

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		

Annex A

Nomogram and formula for calculation of relative centrifugal force (RCF) in the speed of rotation (RPM) depending of the rotor diameter



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