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For research use only

Osteoporosis REAL-TIME PCR Genotyping Kit

REF **R1-H944-N3/4EU**

Package: N (bulk solution)

General information

Intended use:
The **Osteoporosis REAL-TIME PCR Genotyping Kit** is intended for detection and allelic discrimination of genetic polymorphisms associated with inherited risk of osteoporosis and bones fractures.
The **Osteoporosis 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 **Osteoporosis 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).

Kit contents:

Reagent	Organoleptic parameters	Quantity	
PCR-mix:	Colorless transparent liquid		
1. COL1A1: -1997 C>A		960 µL	1 tube
2. COL1A1: 1546 (6252) G>T [Sp1 S>s]		960 µL	1 tube
3. CYP19A1: A>G [rs2414096]		960 µL	1 tube
4. CYP19A1: C>T [rs936306]		960 µL	1 tube
5. ESR1: -397 T>C [PvuII]		960 µL	1 tube
6. ESR1: -351 G>A [XbaI]		960 µL	1 tube
7. IL6: -174 G>C		960 µL	1 tube
8. LRP5: 1999 G>A (Val667Met)		960 µL	1 tube
9. LRP5: 3989 C>T (Ala1330Val)		960 µL	1 tube
10. RANKL: C>T [rs9594738]		960 µL	1 tube
11. RANKL: C>T [rs9594759]		960 µL	1 tube
12. TNFRSF11B (OPG): 245 A>C		960 µL	1 tube
13. TNFRSF11B (OPG): A>G [rs4355801]		960 µL	1 tube
14. TNFRSF11B (OPG): 163 (160) T>C		960 µL	1 tube
15. VDR: 283 A>G (BsmI)		960 µL	1 tube
16. VDR: 2 A>G (Lys2Arg) [FokI]		960 µL	1 tube
PCR-buffer	Colorless transparent liquid	3.84 mL	2 vials
Taq-AT-polymerase	Colorless transparent viscous liquid	192 µL	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	7.68 mL	2 vials

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

Dye label detection channels corresponding to allelic variants and IC

PCR-mix	Fam	Hex	Rox	Cy5	Cy5.5
COL1A1: -1997 C>A	C	A	-	IC	-
COL1A1: 1546 (6252) G>T [Sp1 S>s]	G	T	-	IC	-
CYP19A1: A>G [rs2414096]	A	G	-	IC	-
CYP19A1: C>T [rs936306]	C	T	-	IC	-
ESR1: -397 T>C [PvuII]	T	C	-	IC	-
ESR1: -351 G>A [XbaI]	A	G	-	IC	-
IL6: -174 G>C	G	C	-	IC	-
LRP5: 1999 G>A (Val667Met)	G	A	-	IC	-
LRP5: 3989 C>T (Ala1330Val)	C	T	-	IC	-
RANKL: C>T [rs9594738]	C	T	-	IC	-
RANKL: C>T [rs9594759]	T	C	-	IC	-
TNFRSF11B (OPG): 245 A>C	A	C	-	IC	-
TNFRSF11B (OPG): A>G [rs4355801]	A	G	-	IC	-
TNFRSF11B (OPG): 163 (160) T>C	T	C	-	IC	-
VDR: 283 A>G (BsmI)	G	A	-	IC	-
VDR: 2 A>G (Lys2Arg) [FokI]	A	G	-	IC	-

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

	COL1A1: -1997 C>A	COL1A1: 1546 (6252) G>T [Sp1 S>s]	CYP19A1: A>G [rs2414096]	CYP19A1: C>T [rs936306]	ESR1: -397 T>C [PvuII]	ESR1: -351 G>A [XbaI]	IL6: -174 G>C	LRP5: 1999 G>A (Val667Met)	LRP5: 3989 C>T (Ala1330Val)	RANKL: C>T [rs9594738]	RANKL: C>T [rs9594759]	TNFRSF11B (OPG): 245 A>C	TNFRSF11B (OPG): A>G [rs4355801]	TNFRSF11B (OPG): 163 (160) T>C	VDR: 283 A>G (BsmI)	VDR: 2 A>G (Lys2Arg) [FokI]
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.

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.
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 (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 instrument[1]. Add corresponding test[2], 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>32.0) will be analyzed as N/A (uncertain result).

Storage, shipping and handling requirements

All components of the **Osteoporosis 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.

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)

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

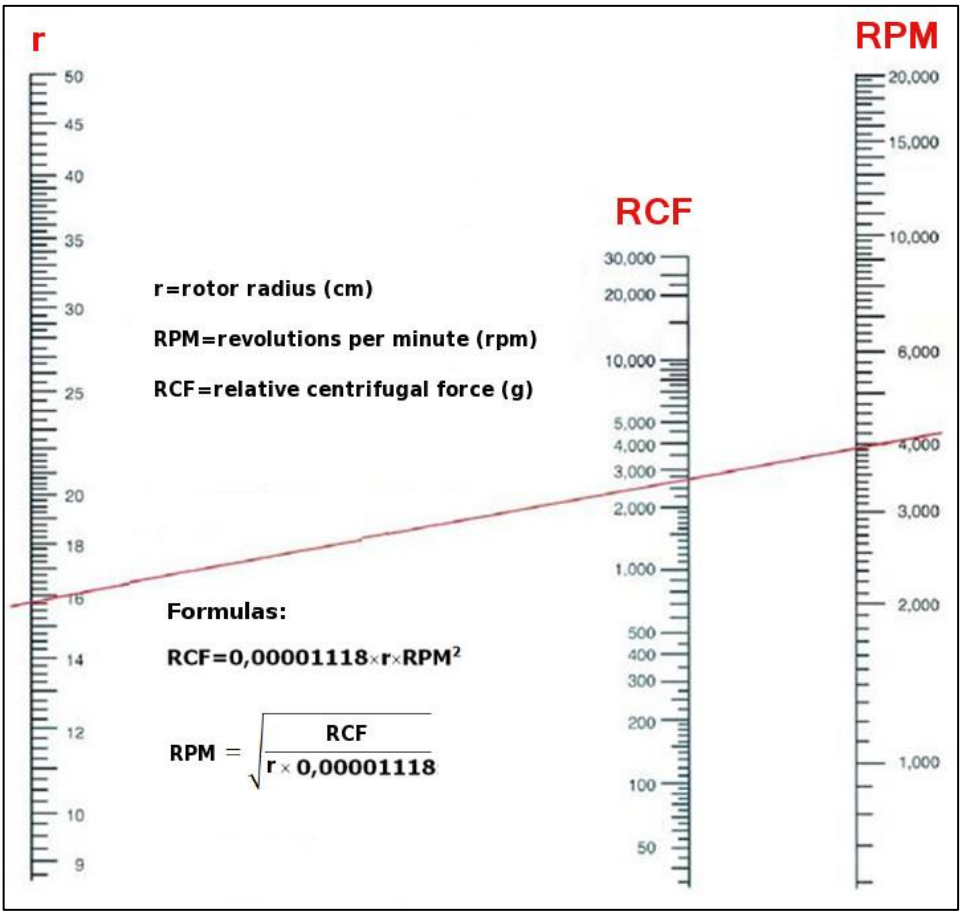
Key to symbols

	Temperature limit		Consult instructions for use	REF	Catalogue number
	Use-by date		Manufacturer	LOT	Batch code
	Date of manufacture		Contains sufficient for <n> tests		Keep away from sunlight
	Caution	NON STERILE	Non-sterile		

[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.

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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