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For research use only

ParodontoScreen REAL-TIME PCR Detection Kit

REF **R1-P808-S3/5EU**

General information

Intended use:
ParodontoScreen REAL-TIME PCR Detection Kit is designed for detection of opportunistic microorganisms inhabiting human oral cavity.
ParodontoScreen REAL-TIME PCR Detection Kit can be used in scientific research practice.

Method:
PCR, qualitative analysis.

Samples:
Crevicular fluids, dental plaque.

DNA extraction:
The DNA-Technology's **PREP-GS PLUS** and **PREP-NA PLUS** extraction kits are recommended.

Features:
PCR-mix contains Internal Control (IC). IC is intended for assessment of PCR run correctness.
Assay includes Sample Intake Control (SIC), which is intended for extraction quality assessment as well as for evaluation of DNA sufficiency for obtaining reliable result.
Positive control (C+) supplied with the kit is intended for specific PCR assessment.
Assay includes Marker, which is intended to control the correct position of a strip in thermal unit.
We also recommend including negative control (C-), which is not supplied but is very helpful for contamination control purposes. Use deionized water or sterile buffered saline instead of negative control, starting from extraction step.

Devices:
The automatic analysis for **ParodontoScreen Real-Time PCR Detection Kit** is available on "DNA-Technology" made DTlite[1] and DTprime[2] REAL-TIME Thermal Cyclers; the latest version of the software is available for download at https://www.dna-technology.com/software.

Hands-on time (including sample preparation):
4 hours.

The number of tests:
24 (including one positive control and one negative control in each run).

Kit contents:

Reagent	Organoleptic parameters	Quantity	
1. Paraffin sealed PCR-mix	Colorless or blue transparent liquid under white wax layer	20 µL in each	24 8-tubes strips
2. Taq-polymerase solution	Colorless transparent liquid	500 µL	4 tubes
3. Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	4 tubes
4. Positive control	Colorless transparent liquid	150 µL	1 tube
Associated accessories:			
Strip's caps			24 8-caps

Strip content, colour codes and detection channels

Tube No.	Cannel			Color code
	Fam	Hex	Rox	
1	Total Bacterial Genome Count (TBGC)	IC	-	Blue
2	*Actinobacillus actinomycetemcomitans*	IC	-	Colorless
3	*Porphyromonas gingivalis*	IC	-	
4	*Prevotella intermedia*	IC	-	
5	*Tannerella forsythensis (Bacteroides forsythus)*	IC	Marker	
6	*Treponema denticola*	IC	-	
7	*Candida albicans*	IC	-	
8	SIC	IC	-	

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

Procedure

1 PCR amplification

The reagents and tubes should be kept away from direct sun light!
Strictly observe the completeness of the strips and caps to them. Do not use the caps for the strips of the other kits!

1.1 Mark the required number of strips for all samples and controls to be tested (one for each sample, one for "C-" and one for "C+").

Example. If you need to test 2 samples, mark 4 strips (one for each sample, one for "C-", one for "C+").

Sample 1	Strip 1
Sample 2	Strip 2
"C-"	Strip 3
"C+"	Strip 4

1.2 Vortex Taq-polymerase solution thoroughly (3-5 s), then spin briefly (1-3 s).
1.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.
1.4 Add one drop (~20 µL) of mineral oil into each tube. Close strips.
1.5 Vortex the tubes with samples, "C-" and "C+" for 3-5 s and spin down drops for 1-3 s.

1. In case of using **PREP-GS PLUS** extraction kits. After vortexing centrifuge the tubes with DNA preparation at RCF(g) 16000 for one minute at room temperature (from 18 °C to 25 °C) to precipitate the sorbent. If, after extraction, the supernatant containing the extracted DNA was transferred to new tubes, centrifugation is carried out for 1-3 s in a vortex mixer.

2. Open the strip, add DNA sample (or control sample), then close the strip before proceeding to the next strip to prevent contamination. Use filter tips. Close strips tightly.

1.6 Add 5.0 µL of the DNA sample into corresponding strips. Avoid paraffin layer break. Do not add DNA into the "C-", "C+" strips.
1.7 Add 5.0 µL of negative control sample (C-) which passed the whole DNA extraction procedure into the corresponding strip. Add 5.0 µL of positive control sample (C+) into the corresponding strip. Avoid paraffin layer break.
1.8 Vortex strips for 1–3 s to collect drops.
1.9 Set the strips into real-time PCR thermal cycler.
Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and IDs of the samples, positive and negative controls. Specify the position of the strips in the thermal unit (see 1.9) and run PCR.

2 Data collection and data analysis

Registration of the PCR results is held in automatic mode.

Interpretation of the results is carried out in accordance with Table 1.

If the value for SIC is less than 2.5/, the sample considered containing insufficient amount of DNA for obtaining reliable result.

Table 1. Interpretation of PCR results based on relative quantity of microorganisms at different clinical evidences and expressed as logarithm (Lg) of genome equivalents per sample.

№	Microorganism	Norm	The severity of periodontitis	
			Light/Moderate	Severe
1	TBGC	<6.5	≥6.5	>7.5
2	*Actinobacillus actinomycetemcomitans*	<4.0	≥4.0	>5.0
3	*Porphyromonas gingivalis*	<5.0	≥5.0	>6.0
4	*Prevotella intermedia*	<4.5	≥4.5	>6.0
5	*Tannerella forsythensis (Bacteroides forsythus)*	<5.0	≥5.0	>5.5
6	*Treponema denticola*	<3.5	≥3.5	>5.0
7	*Candida albicans*	<4.5	≥4.5	>6.0

[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 **ParodontoScreen REAL-TIME PCR Detection Kit** must be stored at the temperatures from 2 °C to 8 °C throughout the shelf life of the kit.

Paraffin sealed PCR-mix should be stored at the temperatures from 2 °C to 8 °C and out of light throughout the shelf life of the kit. Excessive temperature and light can be detrimental to product performance.

Transportation of the kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermobox corresponding to the storage conditions of the components included in the kit.

Transportation of the kit is allowed in thermobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but for no longer than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

Kits transported with violation of temperature conditions shall not be used.

Shelf-life – 9 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		Do not reuse