DNA-TECHNOLOGY

For research purposes only

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ParodontoScreen REAL-TIME PCR Detection Kit

REF R1-P808-S3/5EU

General information

Intended use:
ParodontoScreen REAL-TIME PCR Detection Kit is intended for detection of opportunistic microorganisms inhabiting human oral cavity.

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.

Controls:
PCR-Mix contains Internal Control plasmid (IC). IC is intended for assessment of PCR setup correctness.
Assay includes Sample Intake Control (SIC), which is intended for extraction quality assessment as well as for evaluation of sufficiency of sample for obtaining reliable result.
Positive control plasmid ("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 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 **ParodontoScreen Real-Time PCR Detection Kit** is available on "DNA-Technology" made DTlite[1] and DTprime[2] REAL-TIME Thermal Cyclers; software version is not lower than 7.6.5.12; the current version of the software is available for download at http://www.dna-technology.ru/eng/support/.

Overall time needed to perform the analysis (including sample preparation procedure):
4 hours.

The number of tests:
24

Kit contents:

Reagent	Quantity	
• Paraffin sealed PCR-mix	20 µL	24 8-tube strips
• Taq-polymerase solution	480 µL	4 tubes
• Mineral oil	960 µL	4 tubes
• Positive control (C+)	150 µL	1 tube

Strip content, colour codes and detection channels

№ of tube in a strip	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

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 the Taq-polymerase solution thoroughly (3-5 sec), then spin briefly (1-3 sec).
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 tubes tightly.
1.5 Add 5.0 µL of the DNA sample into corresponding PCR-tubes. Open the tube, add DNA sample, then close the tube before proceeding to the next cDNA sample to prevent contamination. Use filter tips. Do not add DNA into the “C-”, “C+”.
1.6 Add 5.0 µL of negative control sample (“C-”) which passed whole DNA extraction procedure into corresponding strip. Add 5.0 µL of positive control sample (“C+”) into corresponding strip. Avoid paraffin layer break.
1.7 Vortex strips for 1–3 seconds to collect drops.
1.8 Set the strips to real-time PCR thermal cycler.
1.9 Launch the RealTime_PCR application in “Device handling” mode. Upload ini file «Parodont.ini» before the first run.
In subsequent runs add the “Parodont” test to the protocol, specify the number and ID’s of the samples, specify the position of the tubes in the thermal unit (p.1.8) and run PCR.

2 The PCR and post-PCR analysis operated by software and 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

Shipping and storage requirements

All kit components must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance.
Shelf-life – 9 months since the date of production.

Contact our customer service department regarding issues of quality of **ParodontoScreen REAL-TIME PCR Detection Kit**:
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