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For research use only

Sample Intake Control REAL-TIME PCR Kit

REF **R1-P805-23/9EU**
R1-P805-S3/9EU

General information

Intended use:
Sample Intake Control REAL-TIME PCR Kit is intended for detection and approximate quantification of human genomic DNA in biological samples. The Kit is recommended to use:
- for avoiding errors at preanalytical stage during analysis of biological material, containing human epithelial cells (sample intake control by a clinician (SIC));
- for estimation of the amount of human genomic DNA.

Sample Intake Control REAL-TIME PCR Kit can be used in scientific research practice.

Method:
PCR, qualitative analysis.

Samples:
Epithelial cell scrapes from urethra, cervical canal, posterior vaginal vault, posterior nasopharynx wall and other mucosa tissues, cells precipitate from urina, conjunctiva swab, whole blood, etc.

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

Features:
PCR-mix contains Internal Control plasmid (IC). IC is intended for assessment of PCR setup correctness.
Positive control plasmid (C+) supplied with the kit is intended for specific PCR assessment.
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 **Sample Intake Control REAL-TIME PCR 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.

The **Sample Intake Control REAL-TIME PCR Kit** is also approved for use with iCycler iQ5 (Bio-Rad Laboratories) real-time thermal cyclers.

Time of analysis (excluding sample preparation procedure):
2 hours.

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

Kit contents:

Reagent	Organoleptic parameters	Quantity	
1. Paraffin sealed PCR-mix	Colorless transparent liquid under white wax layer	20 µL in each	96 tubes or 12 8-tubes strips
2. Taq-polymerase solution	Colorless transparent liquid	500 µL in each	2 tubes
3. Mineral oil	Colorless transparent viscous oily liquid	1.0 mL in each	2 tubes
4. Positive control	Colorless transparent liquid	150 µL	1 tube
Associated accessories:			
Strip's caps [3]			12 8-caps

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

[3] - for detection kit packaged in strips REF R1-P805-S3/9EU

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
Sample Intake Control	IC	-	-	-

Procedure

1 DNA extraction

DNA extraction is carried out in accordance with the instructions to the used DNA extraction kit. "DNA-Technology's" **PREP-RAPID**, **PREP-RAPID Genetics**, **PREP-GS**, **PREP-GS PLUS**, **PREP-GS Genetics**, **PREP-NA**, **PREP-NA PLUS** extraction kits are recommended.

⚠ Regardless of the used DNA extraction kit perform each step of DNA isolation procedure for negative control (C-) in parallel with test samples.

2 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!

2.1 Mark the required number of the tubes with paraffin sealed PCR-mix considering samples, negative control (C-) and positive control (C+).

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

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

2.2 Vortex the Taq-polymerase solution thoroughly (3-5 s), then spin briefly (1-3 s).
2.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.
2.4 Add one drop (~20 µL) of mineral oil into each tube. Close tubes.
2.5 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, PREP-GS Genetics** and **PREP-GS PLUS** extraction kits. 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.

2. Open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next strip to prevent contamination. Use filter tips. Close tubes/strips tightly.

2.6 Add 5.0 µL of the DNA sample into corresponding PCR-tubes. Avoid paraffin layer break. Do not add DNA into the "C-", "C+" tubes.
2.7 Add 5.0 µL of negative control sample (C-) which passed whole DNA extraction procedure into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.
2.8 Spin tubes/strips for 1–3 s to collect drops.
2.9 Set the tubes/strips to real-time PCR thermal cycler.
2.10 For DTlite and DTprime REAL-TIME Thermal Cyclers: Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 2.9) and run PCR (see Table 1).

For iCycler iQ5 thermal cycler: Turn on the device and the power supply of the device's optical part, leave to heat for 30 minutes. Run Software Bio-Rad iQ5. Create and save a new protocol when the given type of the test for the first time. In subsequent runs select the saved protocol, install configuration of the plate (file with data of the sample ID's and their position in the plate) and run PCR considering the volume of reaction mix 35 µL (see Table 2).

2 Data collection and data analysis

Registration and analysis of the PCR results are carried out in accordance with the instructions to the device. Interpretation of the results is carried out taking into account the Cp values on specificity channel (channel Fam) and internal control channel (channel Hex) in accordance with Tables 3 and 4.

[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.

Table 1. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	min	sec	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	10[1]	…	…	Holding		Holding
[1] – holding at 25°C is allowed						

Table 2. The PCR program for iCycler iQ5 thermal cycler (the switch "Use persistent well factor" must be activated)

Cycle	Repeats	Step	Dwell time	Setpoint, °C	PCR/Melt Data Acquisition
1	1	1	1 min	80	
		2	1 min 30sec	94	
2	5	1	30 sec	94	
		2	45 sec	64	
3	45	1	10 sec	94	
		2	45 sec	64	Real-Time
4	…	…	…	10	Storage

Table 3. Interpretation of PCR results for DTlite and DTprime Thermal Cyclers

Cp on the channel Fam	Cp on the channel Hex	Interpretation	Amount of genomic DNA per reaction, ng
<23	Not considered	Human DNA presents in **sufficient** amount	>750
23-32	Not considered		750-1.0
32-38	Not considered	Human DNA presents in sufficient amount (**excluding genotyping**)	1.0-0.01
>38	27-32	Human DNA presents in **insufficient** amount	<0.01
>38	>32	Possibly, DNA preparation contains PCR inhibitors	<0.01
Not defined	27-32	Human DNA is absent or presents in trace amounts	-
Not defined	Not defined or >32	Unreliable result	-

Table 4. Interpretation of PCR results for iCycler iQ5 thermal cycler

Cp on the channel Fam	Cp on the channel Hex	Interpretation	Amount of genomic DNA per reaction, ng
<18	Not considered	Human DNA presents in **sufficient** amount	>750
18-27	Not considered		750-1.0
27-33	Not considered	Human DNA presents in sufficient amount (**excluding genotyping**)	1.0-0.01
>33	22-27	Human DNA presents in **insufficient** amount	<0.01
>33	>27	Possibly, DNA preparation contains PCR inhibitors	<0.01
Not defined	22-27	Human DNA is absent or presents in trace amounts	-
Not defined	Not defined or >27	Unreliable result	-

Storage, shipping and handling requirements

All components of the **Sample Intake Control REAL-TIME PCR Kit** must be stored at the temperatures from 2 °C to 8 °C during the storage period.

Paraffin-sealed PCR-mix should be stored at the 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.

Transportation can be held by all types of roofed transport at temperatures between 2 °C and 8 °C over the transportation.

Transportation is allowed at the temperature from 0 °C to 24 °C for 72 hours.

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

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)

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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