



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.

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.

Controls:

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 DT-322, DTlite¹, DTprime² and DT-96 REAL-TIME Thermal Cyclers; software version is not lower than 7.3; the current version of the software is available for download at <http://www.dna-technology.ru/eng/support/>.

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



Please enquire DNA-Technology company's representative about compatibility of third-party Real-time instruments.

Overall time needed to perform the analysis (excluding sample preparation procedure):

2 hours.

The number of tests:

96

¹ - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments.

² - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments.

Kit contents:

Reagent	Quantity	
Paraffin sealed PCR-mix	20 µL	96 separate tubes or 12 8-tubes strips
Taq-polymerase solution	500 µL	2 tubes
Mineral oil	1.0 mL	2 tubes
Positive control	150 µL	1 tube

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 DNA extraction kits are recommended.

Please enquire DNA-Technology company's representative about compatibility of third-party DNA extraction kits with Sample Intake Control REAL-TIME PCR Kit.



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

- 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 sec), then spin briefly (1-3 sec).
- 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 tightly.
- 2.5 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 DNA sample to prevent contamination. Use filter tips. Do not add DNA into the "C-", "C+" tubes.
- 2.6 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.
- 2.7 Spin tubes for 1–3 seconds to collect drops.
- 2.8 Set the tubes to real-time PCR thermal cycler.
- 2.9 For DT-322, DTlite, DTprime and DT-96 devices
- 2.9.1 Launch RealTime_PCR software and choose the Device operation mode. Create and save a new test if you do this test for the first time. In subsequent runs add the "SIC" test to the protocol, specify the number and ID's of the samples, specify the position of the tubes in the thermal unit (2.8) and run PCR (see Table 1 for reference).
- 2.10 For iCycler iQ5 device
- 2.10.1 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 for reference).

3 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.

Table 1 - DT-322, DTlite, DTprime and DT-96 cycling program

Nº of block	Temperature, °C	min	sec	Number of cycles	Optical data collection mode	Type of block
1	80.0	0	30	1		Cycle
	94.0	1	30			
2	94.0	0	30	5		Cycle
	64.0	0	15		√	
3	94.0	0	10	45		Cycle
	64.0	0	15		√	
4	94.0	0	5		1	Cycle
5	10.0	Storage		Storage

Table 2 - iCycler iQ5 cycling program (the switch "Use persistent well factor" must be activated)

Cycle	Repeats	Step	Dwell Time	Setpoint, °C	PCR/Melt Data Acquisition
1	1	1	1:00	80	
		2	1:30	94	
2	5	1	0:30	94	
		2	0:45	64	
3	45	1	0:10	94	Real-Time
		2	0:45	64	
4				10	Storage



When using other thermal cyclers please enquire company's representative about cycling program.

Table 3 - Interpretation of PCR results for DT-322, DTlite, DTprime and DT-96


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


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 kit components must be stored 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.

 Paraffin-sealed PCR-mix should be stored in a dark place at the temperatures between 2 °C and 8 °C during the storage period.

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.

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:











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Key to symbols

	Temperature limitation		Consult instructions for use		Catalogue number
	Expiration date		Manufacturer		Batch code
	Date of manufacture		Number of tests		Do not expose to sunlight
	Caution				