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For research use only

INSTRUCTION FOR USE
Streptococcus pyogenes REAL-TIME PCR Detection Kit

REF R1-P402-23/4EU
R1-P402-S3/4EU

General information

Intended use:

The **Streptococcus pyogenes REAL-TIME PCR Detection Kit** is designed to detect *Streptococcus pyogenes* DNA in human biological samples by real-time PCR.

The kit is intended for research use only.

Method:

Real-time polymerase chain reaction; qualitative detection.

DNA extraction:

The "DNA-Technology" **PREP-NA**, **PREP-GS** and **PREP-RAPID** extraction kits are recommended. Some types of the samples must be pretreated (refer to the corresponding instruction for use).

⚠ Use only **PREP-NA** and **PREP-GS** kits for DNA extraction from biopsy samples, phlegm, bronchoalveolar lavage, gastric fluid and faeces.

We do not recommend **PREP-RAPID DNA Extraction Kit** for DNA extraction from male urogenital swabs.

Features:

PCR-mix contains an internal control (IC). IC is intended for PCR quality and sufficiency of DNA assurance.

Positive control plasmid (C+) supplied with the kit is intended for specific PCR assessment.

We also recommend including in the test 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 **Real-Time PCR Detection Kits** is available on "DNA-Technology" made DTlite[1], DTprime[2] Thermal Cyclers; the latest version of the software is available for download at https://www.dna-technology.com/software or Bio-Rad Laboratories iCycler iQ thermal cyclers.

Time of analysis (excluding sample preparation procedure):

from 1.5 h.

The number of tests:

48/96 (including one positive control and one negative control in each run).

Kits content

Reagent	Organoleptic parameters	Quantity	
		48 tests	96 tests
1. Paraffin sealed PCR-mix	Colorless transparent liquid under white wax layer	48 tubes or 6 8-tube-strips (20 µL in each tube)	96 tubes or 12 8-tube-strips (20 µL in each tube)
2. Taq-polymerase solution	Colorless transparent liquid	1 tube (total volume 500 µL)	2 tubes (500 µL in each tube)
3. Mineral oil	Colorless transparent viscous oily liquid	1 tube (total volume 1.0 mL)	2 tubes (1.0 mL in each tube)
4. Positive control[3]	Colorless transparent liquid	1 tube (total volume 75 µL)	1 tube (total volume 150 µL)
Associated accessories: Strip's caps [4]		6 8-caps	12 8-caps

[1] - 4S1, 4S2, 5S2, 6S1, 6S2 models.
[2] - 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 models.
[3] - marking as C+ is allowed.
[4] - for detection kit packaged in strips.

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
Streptococcus pyogenes	IC	-	-	-

Procedure

1 PCR amplification

The reagents and tubes should be kept away from direct sun light.

In case of using tubes in strips, 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 tubes with paraffin sealed PCR-mix for test samples, negative control (C-) and positive control (C+).

Example. If you need to test 5 samples, mark 5 tubes for samples, 1 tube for "C-" and 1 tube for "C+". Total number of tubes - 7.

1.2 Mix the 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 tubes.

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 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 on 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 strips to prevent contamination. Use filter tips.

1.6 Add 5.0 µL of DNA sample into corresponding PCR-tubes. Avoid paraffin layer break. Use filter tips. Do not add DNA into the "C-", "C+" tubes.

1.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedures and positive control (C+) into corresponding tubes. Avoid paraffin layer break.

1.8 Spin the tubes for 1–3 s.

1.9 Set the tubes to Real-time PCR Thermal Cycler.

1.10 For DTlite and DTprime thermal cyclers:

Launch the operating software for DT instrument[5]. Add corresponding test[6], specify the number and IDs of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (1.9) and run PCR (see table 1).

For iCycler iQ thermal cyclers:

Switch on the thermal and optical units of the device and let it warm up for 30 min. Launch iCycler (or Bio-Rad iQ5) software. Create and save new protocol if you do this protocol for the first time. In subsequent runs add the saved protocol, configure the plate (create the file with the data on samples ID's and position) and run PCR considering the total PCR reaction volume equal to 35 µL (see tables 2, 3).

[5] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[6] 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, ºC	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80.0	0	30	1		Cycle
	94.0	1	30			
2	94.0	0	30	5		Cycle
	64.0	0	15		v	
3	94.0	0	10	45		Cycle
	64.0	0	15		v	
4	94.0	0	5	1		Cycle
5	10.0[7]	…	…	…		Holding

Table 2. The PCR program for iCycler iQ5 thermal cyclers (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80.0	
		2	1 min 30 sec	94.0	
2	5				
		1	30 sec	94.0	
		2	45 sec	64.0	
3	45				
		1	10 sec	94.0	
		2	45 sec	64.0	Real Time
4	…	…	…	10.0	Storage

Table 3. The PCR program for iCycler iQ thermal cyclers (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80.0	
		2	1 min 30 sec	94.0	
2	5				
		1	30 sec	94.0	
		2	45 sec	64.0	
3	2				
		1	30 sec	80.0	Real Time
PCR program					
4	45				
		1	10 sec	94.0	
		2	45 sec	64.0	Real Time
5	…	…	…	10.0	Storage

2 Data collection and data analysis

Registration and interpretation of the PCR results is held in automatic mode.

[7] Holding at 25 ºC is allowed

Storage, shipping and handling requirements

All components of the kits should be stored at the temperatures from 2 °C to 8 °C during the storage period.

Paraffin sealed PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

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 during all storage period of the kit.

Shelf life – 12 months since the date of production in compliance with all transportation, storage and operation conditions.

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		

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