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For research use only

Bacillus anthracis REAL-TIME PCR Detection Kit

REF **R1-P702-23/4EU**
R1-P702-S3/4EU

General information

Intended use:
Bacillus anthracis REAL-TIME PCR Detection Kit is intended for DNA amplification and detection of pagA gene (plasmid pXO1) and of capC gene (plasmid pXO2) of Bacillus anthracis.
Bacillus anthracis REAL-TIME PCR Detection Kit can be used in scientific research practice.

Method:
PCR, qualitative analysis.

Samples:
Biological material, washings from environmental objects.

DNA extraction:
The "DNA-Technology" **PREP-GS DNA Extraction Kit** is recommended.

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

Devices:
The automatic analysis for **Bacillus anthracis 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.
The **Bacillus anthracis REAL-TIME PCR Detection Kit** is also approved for use with iCycler (Bio-Rad Laboratories) real-time thermal cyclers.

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

The number of tests:
48 (including positive and negative control samples in each run)

Kit contents:

Reagent	Organoleptic parameters	Quantity	
• Paraffin sealed PCR-mix "*Bacillus anthracis* pagA"	Colorless transparent liquid under white wax layer	20 µL in each	48 individual tubes or 6 8-tubes strips
• Paraffin sealed PCR-mix "*Bacillus anthracis* capC"	Colorless transparent liquid under white wax layer	20 µL in each	48 individual tubes or 6 8-tubes strips
• Taq-polymerase solution	Colorless transparent liquid	500 µL	2 tubes
• Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	2 tubes
• Positive control "*Bacillus anthracis* pagA"	Colorless transparent liquid	75 µL	1 tube
• Positive control "*Bacillus anthracis* capC"	Colorless transparent liquid	75 µL	1 tube
Associated accessories: Strip's caps[3]	12 8-caps		

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
Bacillus anthracis pagA	IC	-	-	-
Bacillus anthracis capC	IC	-	-	-

[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-P702-S3/4EU

Procedure

1 DNA extraction

As a result of sample pretreatment, 0.2-0.5 mL of analyzed material (suspension of bacterial cells) should be obtained in 1.5 mL tube. This material can be used for DNA extraction.

⚠ 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. The volume of saline solution should be the same as the sample volume.

2 PCR amplification

⚠ For detection of fragments of pagA gene or capC gene of *Bacillus anthracis* it is necessary to use "*Bacillus anthracis* pagA" or "*Bacillus anthracis* capC" PCR-mixes respectively.

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

Strictly observe the completeness of the strips and caps for 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 for each PCR-mix "*Bacillus anthracis* pagA" and "*Bacillus anthracis* capC", considering samples, negative control "C-" and one positive control for each PCR-mix "C+ *Bacillus anthracis* pagA" and "C+ *Bacillus anthracis* capC".

Example. If you need to test 2 samples, mark two tubes for each sample, two tubes for "C-", one tube for "C+ *Bacillus anthracis* pagA" and one tube for "C+ *Bacillus anthracis* capC". Total number of tubes – 8.

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 and "C-" and "C+" for 3-5 s and spin down the drops by centrifuging on vortex mixer 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 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. Do not add DNA into the "C-", "C+".

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+ *Bacillus anthracis pagA*" into corresponding tube. Add 5.0 µL of positive control sample "C+ *Bacillus anthracis capC*" into corresponding tube. Avoid paraffin layer break.

2.8 Spin down the drops by centrifuging on vortex mixer for 1-3 s.

2.9 Set the tubes/strips to real-time PCR thermal cycler.

2.10 For DTlite and DTprime devices:

Launch the operating software for DT instrument[4]. Add corresponding test[5], 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 device:
Turn on the device and the power supply of the device's optical part, leave to heat for 30 min. Run Software Bio-Rad iQ. 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).

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

№ 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
	62.0	0	15		√	
3	94.0	0	10	45		Cycle
	62.0	0	15		√	
4	10.01	…	…	Holding		Holding
1 – holding at 25°C is allowed						

[4] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[5] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 2. The PCR program for iCycler iQ (Bio-Rad Laboratories)

Cycle	Repeats	Step	Dwell Time	Setpoint, ˚C	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	60 sec	80.0	
		2	1 min 30 sec	94.0	
2	5				
		1	30 sec	94.0	
		2	45 sec	62.0	
3	2				
		1	30 sec	80.0	Real Time
PCR program					
4	45				
		1	10 sec	94.0	
		2	45 sec	62.0	Real Time
5		…	…	10.0	storage

3 Data collection and data analysis

Registration and analysis of the PCR results are carried out in accordance with the instructions to the device.

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.

Paraffin-sealed PCR-mix should be stored in a dark place at the temperatures from 2 °C to 8 °C 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 from 2 °C to 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 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		