DNA-TECHNOLOGY

For research use only

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Bacillus anthracis REAL-TIME PCR Kit

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

General information

Intended use:
***Bacillus anthracis* REAL-TIME PCR Kit** is intended for DNA amplification and detection of pagA gene (plasmid pXO1) and of capC gene (plasmid pXO2) of Bacillus anthracis.
The kit is for research use only.

Method:
PCR, qualitative analysis.

Samples:
Biological material, washings from environmental objects.

DNA extraction:
The "DNA-Technology" PREP-GS extraction kit is recommended for DNA extraction.

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 Kit** is available on "DNA-Technology" made DT-322, DTlite[1], DTprime[2] 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 ***Bacillus anthracis* REAL-TIME PCR 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

Kit contents:

Reagent	Organoleptic parameters	Quantity	
• Paraffin sealed PCR-mix "*Bacillus anthracis* pagA"	Transparent colorless liquid under white wax layer	20 µL in each	48 separate tubes or 6 8-tubes strips
• Paraffin sealed PCR-mix "*Bacillus anthracis* capC"	Transparent colorless liquid under white wax layer	20 µL in each	48 separate tubes or 6 8-tubes strips
• Taq-polymerase solution	Colorless transparent liquid	500 µL	2 tubes
• Mineral oil	Transparent colorless 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

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.

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.

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".
For 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 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 Vortex the tubes with samples and "C-" and "C+" for 3-5 sec and spin down the drops by centrifuging on vortex-microcentrifuge for 1-3 sec.

Open the cap of the tube/strip, add DNA sample, then close the tube/strip before proceeding to the next tube/strip to prevent contamination. Use filter tips.

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-microcentrifuge for 1-3 sec.

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

2.10 For DT-322, DTlite, DTprime and DT-96 devices:
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 test to the protocol, specify the number and ID's of the samples, specify the position of the tubes in the thermal unit (2.9) and run PCR (see Table 1 for reference).

2.11 For ICycler device:
Turn on the device and the power supply of the device's optical part, leave to heat for 30 minutes. 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 for reference).

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

№ 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	10.0	…	…	Storage		Хранение

Table 2 - iCycler cycling program

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. 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 from 2 °C to 8 °C during the storage period.

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 with quality issues of Bacillus anthracis:
Technical support E-mail: hotline@dna-technology.ru,
www.dna-technology.com

Key to symbols

	Temperature limitation		Consult instructions for use	REF	Catalogue number
	Expiration date		Manufacturer	LOT	Batch code
	Date of manufacture		Number of tests		Do not expose to sunlight
	Caution	NON STERILE	Not sterile		Single use

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