DNA-TECHNOLOGY
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Customer service department:
Phone:+7(495)640.16.93,
Phone/Fax: +7(495)640.17.71.
E-mail: hotline@dna-technology.ru,
www.dna-technology.ru

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Borrelia burgdorferi PCR Detection Kit

("Flash", "Real-Time" formats)

General information

Intended use:
Borrelia burgdorferi PCR Detection Kit is intended for detection of *Borrelia burgdorferi* DNA *in vitro* by polymerase chain reaction (PCR) method.

Method:
PCR, qualitative analysis.

Samples:
Human blood plasma, ixodes ticks.

DNA extraction:
The DNA-Technology's PREP-NA extraction kit is recommended.

Features:
PCR-Mix contains an internal control (DNA-IC). IC is intended for PCR quality assurance.
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.
Depending on the method of the results detection PCR amplification kit is available in two formats:
"Flash" is intended for end-point PCR results detection using a fluorescent detector;
"Real-Time" is intended for detection of PCR results in the course of amplification using a detecting thermocycler.

Devices:

"FLASH" format

The DNA-Technology's made Tercyc Conventional PCR thermal cycler; the DNA-Technology's made fluorescent reader "Gene", software version is not lower than 3.3, recommended version is 4.4.0.10 or fluorescent reader "Gene-4", software version is not lower than 4.4.0.8, recommended version is 4.4.0.10.

"Real-Time" format

The automatic analysis for *Borrelia burgdorferi* PCR Detection Kit is available on "DNA-Technology" made DT*lite*[1], DT*prime*[2] and DT-96 REAL-TIME Thermal Cyclers; software version is not lower than 7.3.4.0; the current version of the software is available for download at http://www.dna-technology.ru/eng/support/.
The *Borrelia burgdorferi* PCR Detection Kit is also approved for use with iQ (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 (including sample preparation procedure):
5 hours.

The number of tests:
48[a]/50[b]

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments
[2] - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments
[a] - in the "Real-Time" format kits
[b] - in the "Flash" format kits

Kit contents:

Reagent	Quantity	
Paraffin sealed PCR-mix	20 µL	48[a]/50[b] tubes
Taq-polymerase solution	500 µL	1 tube
PCR buffer (Background)[b]	200 µL	1 tube
Mineral oil	1 mL	1 tube
Positive control	75 µL	1 tube

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
Borrelia burgdorferi DNA	IC	-	-	-

Procedure

1 PCR amplification

1.1 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+").

⚠ When used "Flash" format kit it is necessary to mark two additional tubes with paraffin sealed PCR-mix ("BACKGROUND" tubes) to control the background fluorescence.

Example. If you need to test 2 samples, mark 6 tubes (1 for each sample, 1 for "C-", 1 for "C+", 2 for "BACKGROUND").

Sample 1	Tube 1
Sample 2	Tube 2
"C-"	Tube 3
"C+"	Tube 4
"BACKGROUND"- 1 ("Flash" format only!)	Tube 5
"BACKGROUND"- 2 ("Flash" format only!)	Tube 6

1.2 Vortex the Taq-polymerase solution thoroughly (3-5 sec), then spin briefly (1-3 sec).

1.3 Add 10 µL of Taq-polymerase solution into each tube except "BACKGROUND" tubes. Add 10 µL of PCR buffer into each "BACKGROUND" tube. Avoid paraffin layer break.

1.4 Add one drop (~20 µL) of mineral oil into each tube. Close tubes tightly.

1.5 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+", "BACKGROUND" tubes.

1.6 Add 5.0 µL of the "C-" which passed whole DNA extraction procedures into "C-" and "BACKGROUND" tubes. Add 5.0 µL of the "C+" into corresponding tube. Avoid paraffin layer break.

1.7 Spin tubes briefly at 1000 RPM for 3-5 sec.

1.8 Place the tubes to the detecting thermal cycler or Tercyc thermal cycler.

1.9 FLASH" format
Carry out the PCR considering the reaction volume of 35 µL (see Table 1 for reference).

1.10 "Real-time" format

For DT*lite* and DT*prime* devices:
Launch RealTime_PCR software and choose the Device handling mode. Create and save a new test if you do this test for the first time. In subsequent runs add the "*Borrelia burgdorferi*" test to the protocol, specify the number and ID's of the samples, specify the position of the tubes in the thermal unit (p. 1.8) and run PCR (see Table 2 for reference).

For ICycler IQ device:
Turn on the device and the power supply of the device's optical part, leave to heat for 30 minutes. Run Software iCycler (or Bio-Rad iQ5). Create and save a new protocol when the given type of the test for the first time. In subsequent productions 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 3 for reference).

2 Data collection and data analysis

"Flash" format. Detection of PCR results is carried out using PCR detector "Gene" or "Gene-4" according to the instruction of the device (the threshold values are 1,75–2,10 for a specific product and 2,50 for internal control) or with horizontal gel electrophoresis (see Table 5 and user's guide to the reagent kit for PCR products detection with the gel-electrophoresis method). Interpretation of the results is carried out in accordance with Tables 4, 5.
"Real-Time" format. Detection of PCR results is carried out using DT*lite*, DT*prime* or iCycler iQ5 according to the instruction of the devices or with horizontal gel electrophoresis (see Table 5 and user's guide to the reagent kit for PCR products detection with the gel-electrophoresis method). Interpretation of the results is carried out in accordance with Tables 4, 5.

Table 1.

"Flash" format. Tercyc cycling program. «Precision active regulation» operational algorithm

№ of block	For thermal cyclers with active regulation			Number of cycles
	Temperature, °C	Time		
		min	sek	
1	94.0	1	30	1
2	94.0 64.0 67.0	0 0 0	5 5 5	5
3	94.0 64.0 67.0	0 0 0	1 5 5	40
4	10.0	…		Storage

⚠ When working with Flash PCR detection Kits once prepared and amplified "BACKGROUND" tubes may be used many times at each PCR results detection with reaction tubes from the same lot. "BACKGROUND" tubes can be stored between 2 and 8 °C and out of light for 1 month. During the detection procedure "BACKGROUND" tubes must be room temperature (between 18 and 25 °C), for that take out them from refrigerator 1 hour before detection.

Table 2.

"Real-Time" format. DT*lite* and DT*prime* 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
	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 3.

"Real-Time" format. iCycler iQ (Bio-Rad Laboratories) cycling program.

Cycle	Repeats	Step	Dwell Time	Setpoint, °C	PCR/Melt Data Acquisition
Well dynamic factor readout program (dynamicwf.tmo)					
1	1	1	1 min	80	
		2	1 min 30 sec	94	
2	5	1	30 sec	94	
		2	45 sec	64	
3	2	1	30 sec	80	Real-Time
PCR program					
4	45	1	10 sec	94	
		2	45 sec	64	Real-Time
5				10	Storage

Table 4.

PCR results interpretation.

"Flash" format. Gene results	"Real-time" format		Interpretation
	FAM channel results (specific DNA)	Hex channel results (internal control)	
Analyzed samples			
"+"	Cp/Ct is not specified	Not considered	DNA of *Borrelia burgdorferi* is detected ("+")
"-"	Cp is not specified (N/A for IQ)	Cp/Ct is specified	DNA of *Borrelia burgdorferi* is not detected ("-")
N/A	Cp is not specified (N/A for IQ)	Cp is not specified (N/A for IQ)	Not reliable result (N/A)
Positive control sample			
"+"	Cp/Ct is specified	Not considered	Positive result ("+")
Negative control sample			
"-"	Cp is not specified (N/A for IQ)	Cp/Ct is specified	Negative result ("-")

* - results from "Qualitative analysis" line of DT*lite*, DT*prime* software are shown in the brackets.

Table 5.

PCR results interpretation with horizontal gel electrophoresis

Specific product (335 b.p.)	Internal control (560 b.p)	Interpretation
Analyzed samples		
+	Not considered	DNA of *Borrelia burgdorferi* is detected
-	+	DNA of *Borrelia burgdorferi* is not detected
-	-	Not reliable result
Positive control sample		
+	Not considered	Positive result
Negative control sample		
-	+	Negative result

Storage and handling requirements

All kit components must be stored at temperature between 2 °C and 8 °C out of light during the storage period. The excessive temperature and light can be detrimental to product performance.
Shelf-life – 12 months since the date of production.

Contact our customer service department regarding quality issues with the kit:
8 800 200-75-15 (toll-free call for Russia)
+7 (495) 640-16-93 (chargeable call for CIS and foreign countries).
E-mail: hotline@dna-technology.ru, www.dna-technology.ru.
Address: "DNA-Technology" LLC, 117587, Russia, Moscow, int. ter. Municipal District Chertanovo Severnoye, Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12.

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