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For research use only

Yersinia pestis REAL-TIME PCR Detection Kit

REF **R1-P703-23/4EU**
R1-P703-S3/4EU

General information

Intended use:
Yersinia pestis REAL-TIME PCR Detection Kit is intended for detection of *Yersinia pestis pla* gene DNA (pPst plasmid) and *caf1* gene DNA (pFra plasmid) in the human biological samples, in the samples from dead and sick animals in the ticks, ectoparasites etc. by polymerase chain reaction (PCR) method.
Yersinia pestis REAL-TIME PCR Detection Kit can be used in scientific research practice.

Method:
PCR, qualitative analysis.

Samples:
Discharge of ulcers, punctate from buboes, sputum, blood, faeces, biopsies; samples from dead and sick animals; ticks, ectoparasites etc.

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

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

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

Time of analysis (including sample preparation procedure):
4 hours.

Number of tests:
48 (including positive and negative control samples in each run).

Kit contents:

Reagent	Organoleptic parameters	Quantity	
• Paraffin sealed PCR-mix "*Yersinia pestis pla*"	Colorless transparent liquid under white wax layer	20 µL in each	48 individual tubes or 6 8-tubes strips
• Paraffin sealed PCR-mix "*Yersinia pestis caf1*"	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 "*Yersinia pestis pla*"	Colorless transparent liquid	75 µL	1 tube
• Positive control "*Yersinia pestis caf1*"	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
Yersinia pestis pla	IC	-	-	-
Yersinia pestis caf1	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-P703-S3/4EU

Procedure

1 DNA extraction

As a result of sample preparation procedure must be obtained 0.2-0.5 mL of the material in the plastic 1.5 mL tube. The resulting 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 pla gene or caf1 gene of Yersinia pestis it is necessary to use "Yersinia pestis pla" or "Yersinia pestis caf1" 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 considering samples, negative control (C-) and positive control (C+).

Example. If you need to test 2 samples, mark 8 tubes (2 for each sample, 2 for "C-", 2 for "C+").

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+ Yersinia pestis pla" into corresponding tube. Add 5.0 µL of positive control sample "C+ Yersinia pestis caf1" 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 IQ device:

Turn on the device and the power supply of the device's optical part, leave to heat for 30 min. 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 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
	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[1]	…	…	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
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

3 Data collection and data analysis

Detection of PCR results is carried out using DT*lite*, DT*prime* or iCycler iQ according to the instruction of the devices. Interpretation of the results is carried out in accordance with Table 3.

Table 3. PCR results interpretation

FAM channel results		**HEX channel results (IC)**	**Interpretation**
"*Yersinia pestis pla*" amplification kit	"*Yersinia pestis caf1*" amplification kit		
Analysed samples			
Cp/Ct specified	Cp/Ct specified	Not considered	DNA of *Yersinia pestis* genes *pla* and *caf1* detected
Cp/Ct specified	Cp/Ct not specified	Not considered for "*Yersinia pestis* pla" amplification kit, Cp/Ct specified for "*Yersinia pestis* caf1" amplification kit	DNA of *Yersinia pestis* gene *pla* detected
Cp/Ct not specified	Cp/Ct specified	Not considered for "*Yersinia pestis* caf1" amplification kit, Cp/Ct specified for "*Yersinia pestis* pla" amplification kit	DNA of *Yersinia pestis* gene *caf1* detected
Cp/Ct not specified	Cp/Ct not specified	Cp/Ct specified	DNA of *Yersinia pestis* genes *pla* and *caf1* not detected
Cp/Ct not specified	Cp/Ct not specified	Cp/Ct not specified	Not reliable result
Positive control (C+)			
Cp/Ct specified	Cp/Ct specified	Not considered	Positive result ("+")
Negative control (C-)			
Cp/Ct not specified	Cp/Ct not specified	Cp/Ct specified	Negative result ("-")

Storage, shipping and handling requirements

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

Paraffin-sealed PCR-mix should be stored at the temperatures 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.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

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:

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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