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For research use only

Vibrio cholera REAL-TIME PCR Detection Kit

REF **R1-P701-23/4EU**

General information

Intended use:
Vibrio cholerae PCR REAL-TIME Detection Kit is intended for detection of toxigenic *Vibrio cholerae* strains DNA in bacterial cells suspension by the method of polymerase chain reaction (PCR).
Vibrio cholerae PCR REAL-TIME Detection Kit can be used in scientific research practice.
Method:
PCR, qualitative analysis.

Samples:
Bacterial cells suspension.

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 and sufficiency of DNA assurance. Positive control plasmid (C+) supplied with the kit is intended for specific PCR assessment.
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.

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

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

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

Kit contents:

Reagent	Organoleptic parameters	Quantity	
1. Paraffin sealed PCR-mix	Colorless transparent liquid under white wax layer	20 µL in each	48
2. Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
3. Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
4. Positive control	Colorless transparent liquid	75 µL	1 tube

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
DNA of toxigenic *Vibrio cholera* strains	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 preparation procedure must be obtained 0.2-0.5 mL of the bacterial cells suspension in the plastic 1.5 mL tube. The resulting material can be used for DNA extraction.

2 PCR amplification

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

Strictly observe the completeness of the strips and caps to them. Do not use the caps for the strips of the other kits!

2.1 Mark the required number of tubes with paraffin sealed PCR-mix: 1 tube for each sample to be tested, 1 tube for negative control (C-) and 1 tube for positive control (C+).

Example. If you need to test 2 samples, mark 4 tubes (one for each sample, one for "C-", one for "C+").

Sample 1	Tube 1
Sample 2	Tube 2
"C-"	Tube 3
"C+"	Tube 4

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 the each tube. Avoid paraffin layer break.

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

2.5 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 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. Use filter tips. Close tubes tightly.

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

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+) into corresponding tube. Avoid paraffin layer break.

2.8 Spin tubes for 1-3 s to collect drops.

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

2.10 For DTlite and DTprime REAL-TIME Thermal Cyclers:

Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 2.9) and run PCR (see Table 1).

For iCycler iQ thermal cycler:

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 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

Step	Temperature, °C	min	sec	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	10	…	…	Holding		Holding

[3] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[4] 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	1:00	80	
		2	1:30	94	
2	5	1	0:30	94	
		2	0:45	64	
3	2	1	0:30	80	Real-Time
PCR program					
4	45	1	0:10	94	
		2	0:45	64	Real-Time
5				10	Storage

3 Data collection and data analysis

Detection of PCR results is carried out using DTlite, DTprime or iCycler iQ5 according to the instruction of the devices. Interpretation of the results is carried out in accordance with Table 3.

Table 3. PCR results interpretation

		Interpretation[5]
Analysed samples		
Cp/Ct is specified	Not considered	DNA of toxigenic *Vibrio cholera* strains is detected ("+")
Cp is not specified (N/A for iQ)	Cp/Ct is specified	DNA of toxigenic Vibrio cholera strains is not detected ("-")
Cp is not specified (N/A for iQ)	Cp is not specified (N/A for iQ)	Not reliable result (N/A)
Positive control (C+)		
Cp/Ct is specified	Not considered	Positive result ("+")
Negative control (C-)		
Cp is not specified (N/A for iQ)	Cp/Ct is specified	Negative result ("-")

[5] - in parenthesis the result that represents in the "Qualitative Analysis" field for DTlite and DTprime devices is shown.

Storage, shipping and handling requirements

All components of the ***Vibrio cholerae*** **REAL-TIME PCR Detection Kit** must 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)

+7 (495) 640-16-93 (chargeable call for CIS and foreign countries)

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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		