2021-12

Pathogenic Bacteria Detection Kits

Products Name	Cat. No.	Volume
CP Detection Kit	E-4000	96 tests
Bacillus Detection Kit	E-5000	96 tests
Campylobacter Detection Kit	E-6000	96 tests
Vibrio Detection Kit	E-7000	96 tests

Components:

1) E-4000

1. Multi Premix (2X, with UDG) 96 test x 1 plate
2. Primer Mixture 0.5 mL x 1 tube
3. Control DNA (used like a ladder) 100 ㎕ x 1 tube

2) E-5000, E-6000, E-7000

1. Multi Premix (2X, with UDG) 96 test x 1 plate
2. Primer Mixture 0.5 mL x 1 tube
3. Positive Control DNA 60 ㎕ x 1 tube

Description:

The Pathogenic Bacteria Detection Kits consists of Multi Premix (with UDG) for multiplex PCR, primer mixture and control DNA.
Especially, the UDG (Uracil-DNA Glycosylase) is included in Multi Premix and catalyses the release of free uracil from uracil-containing DNA. This can be protected the cross-contamination of PCR products and false positive.

Target toxins:

Products	Pathogenic bacteria	Target toxin	Size
Clostridium perfringens Detection Kit (E-4000)	Internal control	-	1000 bp
	Clostridium perfringens	cpe	517 bp
		cpa	283 bp
Bacillus cereus Detection Kit (E-5000)	Internal control	-	1000 bp
	Bacillus cereus	Cytk2	644 bp
		nheA	482 bp
		bceT	375 bp
		CER	239 bp
		entFM	190 bp
		hblC	127 bp
Campylobacter Detection Kit (E-6000)	Internal control	-	1000 bp
	Campylobacter jejuni	hipO	389 bp
	Campylobacter coli	glyA	188 bp
Vibrio Detection Kit (E-7000)	Internal control	-	1000 bp
	Vibrio Vulnificus	vvhA	507 bp
	Vibrio cholerae	hlyA	329 bp
	Vibrio parahaemolyticus	tlh	214 bp

Standard reaction protocols

1. Prepare the Bacterial genomic DNA: Prepare for extracted bacterial genomic DNA as template to use in PCR step by variable methods of DNA extraction from clinical samples.

2. Thaw the reagents: Thaw all reagents on ice or lap-top cooler, gently vortex each tube and 8-strip tube to mix the contents thoroughly, the briefly centrifuge to collect the solution and the bottom of each tube. Keep the reagents on ice.

3. Prepare the PCR master mix:

a. Make each component according to the following table in 0.2 mL PCR tube;

Components	Volume
Multi Premix (2X, with UDG)	10 ㎕
Primer Mixture	5 ㎕
extracted DNA	5 ㎕
Total volume of master mix	20 ㎕

b. Close the cap of strip tube, vortex, and then centrifuge briefly to collect the contents to the bottom.

4. Set up and run the real-time PCR instrument:

Step	Temperature	Time	Cycle
UDG Reaction	50 ℃	3 min	1
Pre-denaturation	95 ℃	10 min	1
Denaturation	95 ℃	30 sec	35
Annealing/Extension	68 ℃	45 sec	
Final Extension	72 ℃	5 min	1
Store	12 ℃	∞	-

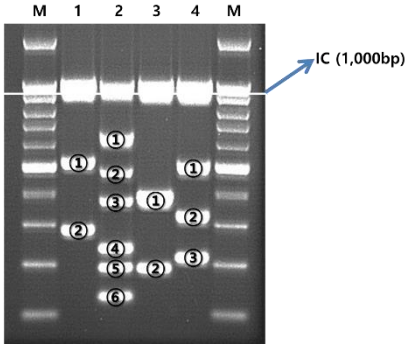
Criterion of Interpretation of test results:

Reaction type	Amplification	Interpretation
internal control (only)	+	Negative
internal control + any toxin	+	Positive
any toxin (only)	+	Positive
internal control, any toxin	-	Suspect result *

* Repeat the PCR with diluted 1/10 and 1/100 of extracted DNA from step 3.

Electrophoresis

- PCR product loading volume: 7 ㎕/5 mm lane width in 1.8% agarose gel
- Control DNA loading volume: 7 ㎕/5 mm lane width in 1.8% agarose gel



Lane M: 100bp DNA Marker (Cat. No. M-1000)
Lane 1: Clostridium perfringens → ① cpe (517bp) ② cpa (283bp)
Lane 2: Bacillus cereus → ① Cytk2 (644bp) ② nheA (482bp) ③ bceT (375bp)
④CER (239bp) ⑤ entFM (190bp) ⑥ hblC (127bp)
Lane 3: Campylobacter jejuni/coli → ① hipO (389bp) ② glyA (188bp)
Lane 4: Vibrio vulnificus/cholera/para → ① vvhA (507bp) ② hlyA (329bp) ③ tlh (214bp)

Research Use Only
Store at -20℃

GENETBIO Inc. Tel.: +82- 0502-523-3484 e-mail: info@genetbio.co.kr homepage: www.genetbio.com