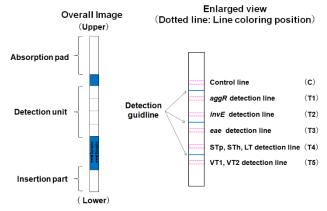


Kaneka Nucleic Acid Chromatography Diarrheagenic E. coli Detection Kit Simple manual

[Component]

1. Test strip	20 strips
2. Chase buffer	20 tests
3. Primer mix1	
(VT1, VT2, eae, invE, aggR)	20 tests
4. Primer mix2	
(LT, STh, STp)	20 tests
5. PCR Mix	40 tests

[Chip appearance]



[How to Use]

DNA extraction process from specimen

Enrich the fecal sample with mEC medium and use the resulting culture solution as the sample to extract DNA with a commercially available DNA extraction kit (Kaneka Easy DNA Extraction Kit Version 2, DNeasy Blood & Tissue Kit - QIAGEN, etc.) DNA extracted/purified using the heat extraction or the phenol/chloroform methods can also be used as a PCR template.

Example) DNA extraction by heat

- (1) Transfer 200µL of the enriched culture broth to another tube and centrifuge at 15,000 rpm for 3 minutes at room temperature.
- (2) After centrifugation, remove the supernatant, add 1 mL sterilized water, and pipette.
- (3) Heat with a heat block at 100° C for 10 minutes.
- (4) Use ice-cooled PCR template.

PCR process

- (1) Thaw Primer Mix 1, Primer Mix 2, and Amplification Enzyme Mix, and mix by inversion or pipetting to homogenize.
- (2) Prepare reaction solutions 1 and 2 on ice according to the table below.

Reactor 1

- todoto.	
1. Primer mix 1	8 µL
2. PCR mix	10 µL
3. Extracted DNA	2 µL

Reactor 2

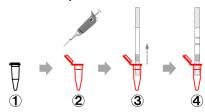
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1. Primer mix 2	8 µL
2. PCR mix	10 µL
3. Extracted DNA	2 μL

(1) Perform PCR according to the table below

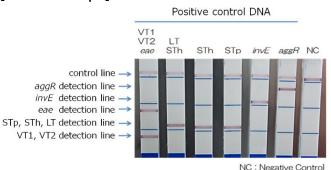
\ /	- 0	
Steps	Temp./Time	# of Times
Step 1	25℃/10min	1
Step 2	94℃/2min	1
	94°C/5sec	
Step 3	56°C/30sec	35
	72°C/20sec	

Detection process

- (1) Prepare reaction solution 1 and 2 after PCR.
 - Add the entire amount of Reaction Solution 2 to the PCR tube containing Reaction Solution 1 (no mixing required).
- (2)Add 140 μL of development buffer to the PCR tube containing reaction solutions 1 and 2 (no mixing required).
- (3) Insert the test strip and leave it at room temperature.
- (4) After 10 minutes, visually check the detection line.



[Detection example]



[Storage Conditions]

• Test strips : Room temp.
• Chase buffer : Room temp.
• Primer mix 1&2 : Room temp. (-20°C)
• PCR mix : Room temp. (-20°C)

[Precautions]

- This product shall be used for research purposes only and not for clinical diagnosis.
- > To get the correct judgment result, please judge 10 minutes after starting and do not evaluate it after that.
- The performance of the test strip will deteriorate when left for a long time in a moisture-containing state. Therefore, after opening the package, be sure to close the container lid and be careful not to absorb moisture.

Kaneka Corporation Medical Solutions Vehicle 2-3-18, NAKANOSHIMA, KITA-KU, OSAKA, 530-8288, JAPAN URL https://www.kaneka-labtest.com