

# Cica Geneus<sup>®</sup> *E. coli* POT KIT



Kanto Reagents



*Escherichia coli* is distributed in environment widely.

In the medical environment, it often causes outbreaks such as food poisoning and nosocomial infection. Then, infection management practices are important.

The PCR-based ORF Typing(POT) is a molecular epidemiological method which can identify international clones and distinguish isolates with strain level. Genotypes of isolates can be obtained using only PCR and agarose gel electrophoresis.

## Characteristics

- This POT method developed by Dr. Suzuki of Fujita health University, it's a molecular epidemiological method which can identify and distinguish isolates with strain-level.
- This POT method can compare the homogeneity between strains by digitizing (replace to POT types) an electrocataphoresis result.
- Nearly the same discriminability as the pulsed field gel electrophoresis (PFGE).
- Required for the examination time is approximately 4 hours.

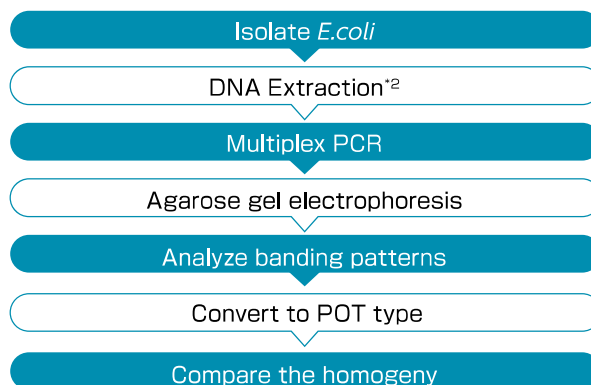
## Composition(30 rxns)

| Reagent                                     | Volume   |
|---|----------|
| A AptaTaq DNA Master(5×Conc.) <sup>*1</sup> | 240 μL×1 |
| B PCR supplement                            | 240 μL×1 |
| C Primer mix α                              | 120 μL×1 |
| D Primer mix β                              | 120 μL×1 |
| E Positive control                          | 240 μL×1 |
| F Loading buffer(6×Conc.)                   | 240 μL×1 |

<sup>\*1</sup> AptaTaq DNA Master(5×Conc.) is the product of Roche Diagnostics K.K.

<sup>\*2</sup> Cica Geneus™ DNA extraction reagent is sold separately.

## Work Flow



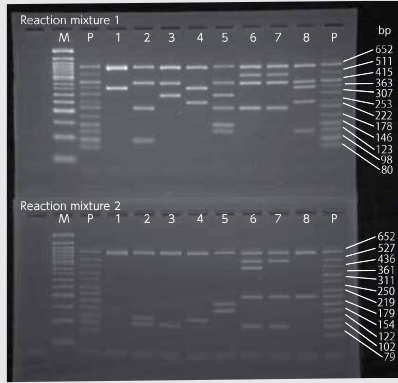


Fig.1 Example of the electrophoretic pattern

M : 50 bp DNA Ladder  
 P : Positive control  
 1 : ATCC 8739      2 : ATCC 11775  
 3 : ATCC 25922    4 : ATCC 27166  
 5 : ATCC 35218    6 : ATCC BAA-2340 (KPC)  
 7 : ATCC BAA-2469 (NDM-1)  
 8 : ATCC 35150 (O-157)

Table.1 Kinds of detection ORF and Amplicon size

|                    | POT No. | Amplicon size (bp) | POT modulus                   | Target domain                   |
|--------------------|---------|--------------------|-------------------------------|---------------------------------|
| Reaction mixture 1 | PCR PC  | 652                | -                             | <i>E. coli</i> Positive control |
|                    | POT 1-1 | 511                | 32                            | Genomic Islet-1                 |
|                    | POT 1-2 | 415                | 16                            | Genomic Islet-2                 |
|                    | POT 1-3 | 363                | 8                             | Genomic Islet-3                 |
|                    | POT 1-4 | 307                | 4                             | Genomic Islet-4                 |
|                    | POT 1-5 | 253                | 2                             | Genomic Islet-5                 |
|                    | POT 1-6 | 222                | 1                             | Genomic Islet-6                 |
|                    | POT 2-1 | 178                | 128                           | <i>bla</i> <sub>CTX-M-2</sub>   |
|                    | POT 2-2 | 146                | 64                            | Genomic Island-1                |
|                    | POT 2-3 | 123                | 32                            | Genomic Island-2                |
| Reaction mixture 2 | PCR PC  | 652                | -                             | <i>E. coli</i> Positive control |
|                    | POT 2-6 | 527                | 4                             | Genomic Islet-7                 |
|                    | POT 2-7 | 436                | 2                             | Genomic Island-5                |
|                    | POT 2-8 | 361                | 1                             | <i>bla</i> <sub>CTX-M-1</sub>   |
|                    | POT 3-1 | 311                | 128                           | Genomic Island-6                |
|                    | POT 3-2 | 250                | 64                            | Genomic Island-7                |
|                    | POT 3-3 | 219                | 32                            | Genomic Islet-8                 |
|                    | POT 3-4 | 179                | 16                            | Genomic Island-8                |
|                    | POT 3-5 | 154                | 8                             | Genomic Island-9                |
|                    | POT 3-6 | 122                | 4                             | Genomic Islet-9                 |
| POT 3-7            | 102     | 2                  | Genomic Island-10             |                                 |
| POT 3-8            | 79      | 1                  | <i>bla</i> <sub>CTX-M-9</sub> |                                 |

- i. Distribution patterns of ORFs are identified by reading electrophoresis banding patterns (Fig.1) .
- ii. POT types of two categories are obtained by input electrophoresis banding patterns into the Excel calculation sheet which can be downloaded from Kanto Chemical Co. Inc web site (Table.1) .
  - i When PCR PC is positive, the sample can confirm that it is *E. coli*.
  - ii The POT 1 value of ST131 which often obtained as ESBL is 49.
  - iii If the value of POT 2 become odd number, the strain has the gene of ESBL, CTX-M-1 group.  
 If it become over 128, the strain has the gene of ESBL, CTX-M-2 group.  
 If the value of POT3 become odd number, the strain has the gene of ESBL, CTX-M-9.
  - iv By comparing POT types, you can objectively estimate homology between strains.
  - v As for the strains obtained from outbreaks, all POT types (1-3) become same.

## Result

| Category | Sample number in Fig.1 |    |    |    |    |    |    |    |
|----------|------------------------|----|----|----|----|----|----|----|
|          | 1                      | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
| POT1     | 8                      | 17 | 20 | 10 | 21 | 49 | 49 | 26 |
| POT2     | 0                      | 16 | 0  | 0  | 96 | 6  | 4  | 32 |
| POT3     | 0                      | 6  | 2  | 4  | 24 | 34 | 34 | 32 |

## Product Information

| Product No. | Product Name                        | Package size | Stored at     |
|-------------|-------------------------------------|--------------|---------------|
| 08362-97    | Cica Geneus® <i>E. coli</i> POT KIT | 30 rxns      | -20 °C~-25 °C |
| 08178-96    | Cica Geneus® DNA Extraction Reagent | 120 rxns     | 2 °C~8 °C     |

### Multiplex PCR kit series (stored at -20 °C ~ -25 °C)

| Product No. | Product Name                                       | Package size |
|-------------|--|--------------|
| 08180-96    | Cica Geneus® Staph POT KIT                         | 120 rxns     |
| 08187-96    | Cica Geneus® Pseudo POT KIT                        | 50 rxns      |
| 08062-96    | Cica Geneus® Acineto POT KIT                       | 30 rxns      |
| 08106-97    | Cica Geneus® <i>C. diff</i> POT KIT                | 30 rxns      |
| 08143-96    | Cica Geneus® AmpC Genotype Detection KIT           | 30 rxns      |
| 08112-96    | Cica Geneus® ESBL Genotype Detection KIT           | 30 rxns      |
| 08158-96    | Cica Geneus® Carbapenemase Genotype Detection KIT2 | 30 rxns      |

### Related reagents

| Product No. | Product Name                | Package size |
|-------------|-----------------------------|--------------|
| 46510-79    | 10XTBE buffer               | 1 L          |
| 46509-79    | 10XTAE buffer               | 1 L          |
| 14575-43    | Ethidium bromide solution   | 10 mL        |
| 01089-23    | Agarose KANTO HC            | 100 g        |
| 01016-96    | AptaTaq DNA Master(5XConc.) | 500 μL       |

● This product is obtaining the patent licensing from Aichi Prefecture and Chubu University.

**Cica** KANTO CHEMICAL CO., INC.  
 REAGENT DIVISION

East Muromachi Mitsui BLDG, 2-1, Nihonbashi Muromachi 2-chome,  
 Chuo-ku, Tokyo, 103-0022, JAPAN

Telephone +81-3-6214-1092  
 Telefax +81-3-3241-1053  
<http://www.kanto.co.jp> E-mail: kanto-61@gms.kanto.co.jp