

Cica Geneus™ Pathogenesis Gene Detection PCR Kit (for Diarrheagenic E.coli) 2



Diarrheagenic *E. coli* is *E. coli* that causes abdominal pain and diarrhea in humans. It is classified into five types and other diarrheagenic *E. coli* based on differences in properties and symptoms. In recent years, there have been frequent cases of food poisoning and outbreaks caused by *Escherichia albertii* (*E. albertii*), closely related to *E. coli*.

This method of this kit is to amplify plural specific genes by Multiplex PCR at the same time, and then to analyze agarose gel electrophoresis banding patterns.

This kit can detect the five main types of diarrheagenic *E. coli*: EHEC/VTEC, EPEC, ETEC, EIEC, and EAggEC, as well as genes related to the pathogenicity of *E. albertii*.

Characteristics

- Using two primer mixes, this kit simultaneously and differentially detects specific genes of *E. coli* and *E. albertii*, and seven virulence factors*¹.

*1 Virulence factors: Shiga toxin (Verotoxin) type 1 and 2 genes (stx1, 2), heat-stable toxin (ST) gene (est), heat-labile toxin (LT) gene (elt), intimin gene (eae), agglutination-associated factor (aggR), and enteroinvasive factor (invE).

- Quantifying detection results allows for clearer interpretation.
- Included are reagents necessary for PCR, including a positive control.
- PCR inhibition can be confirmed based on the internal control detection results.

Composition (50 rxns)

	Reagent	Volume
A	AptaTaq DNA Master (5×Conc.) * ¹	400 μL ×1
B	PCR supplement	700 μL ×1
C	Primer mix1	200 μL ×1
D	Primer mix2	200 μL ×1
E	Positive control	250 μL ×1
F	Loading buffer (6× Conc.)	400 μL ×1

*1 AptaTaq DNA Master (5×Conc.) is the product of Roche Diagnostics K.K.

*2 CicaGeneus™ DNA extraction reagent is sold separately.

Work flow

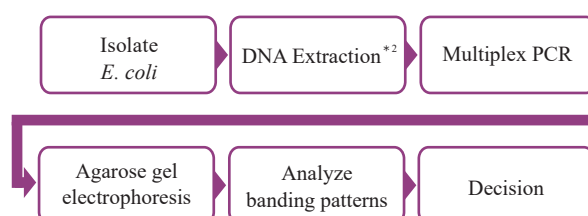
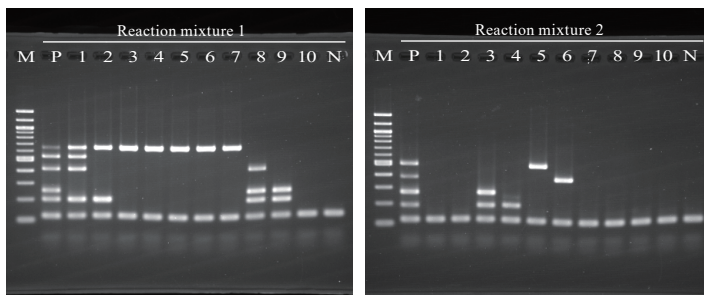


Fig.1 Example of the electrophoretic pattern



M: 100 bp DNA Ladder, P: Positive control, 1: *E. coli* ATCC 35150 (EHEC/VTEC),
 2: *E. coli* TCC 43887 (EPEC), 3: *E. coli* ATCC 35401 (ETEC), 4: *E. coli* NCTC 11603 (ETEC),
 5: *E. coli* (Clinical isolate / EAggEC), 6: *E. coli* ATCC 43893 (EIEC), 7: *E. coli* ATCC 11775 (non-pathogenic),
 8: *E. albertii* (Clinical isolate / stx2f), 9: *E. albertii* LMG 20976, 10: *Citrobacter freundii* ATCC 8090
 N: negative control (TE buffer)

- ① Read banding patterns (Table.1) from two electrophoretic result (Fig.1).
- ② If *uidA* is positive, refer to Table.1 and multiply the result (1 or 0) by the coefficient to calculate the total points. Then, compare the total points with Table.2 to determine the pathotype of *E. coli*.
- ③ If *lysP* is positive, you can estimate that it is *E. albertii*.
- ④ If neither of *uidA* and *lysP* is positive, the bacterial species cannot be determined using this kit.

Result

Category	Sample Number in Fig.1									
	1	2	3	4	5	6	7	8	9	10
Total point	97	1	24	16	2	4	0	65	1	0
Decision	EHEC/VTEC (stx1+ stx2+ eae+)	EPEC (eae+)	ETEC (est+ elt+)	ETEC (est+)	EAggEC (aggR+)	EIEC (invE+)	<i>E. coli</i>	<i>E. albertii</i> (stx2+ eae+)	<i>E. albertii</i> (eae+)	ND

Table.1 Kind of detection genes and Amplicon size

	Coefficient	Amplicon Size (bp)	Target gene
Reaction mixture 1	—	652	<i>uidA</i> (<i>E. coli</i> specific gene)
	32	532	<i>stx1</i>
	64	413	<i>stx2</i>
	—	252	<i>lysP</i> (<i>E. albertii</i> specific gene)
	1	199	<i>eae</i>
	—	120	Internal control
Reaction mixture 2	2	499	<i>aggR</i>
	4	379	<i>invE</i>
	8	270	<i>elt</i>
	16	191	<i>est</i>
	—	120	Internal control

This kit

* detects Shiga toxin gene carried by *Shigella* as stx1.

* detects *stx2f* variant carried by *E. albertii* as stx2.

* detects the *eae* gene carried by the *Citrobacter*.

* detects both porcine type (STp) and human type (STh) of *est*.

* cannot distinguish between *Shigella* and *E. coli* because it also detects *uidA*.

Table.2 Total point and Kind of pathogenesis

Total point	Kind of pathogenesis (<i>E. coli</i>)
0	<i>E. coli</i> other than EHEC/VTEC, EPEC, ETEC, EIEC, EAggEC
1	EPEC (eae+)
2	EAggEC (aggR+)
4~7	EIEC (invE+)
8~15	ETEC (elt+)
16~23	ETEC (est+)
24~31	ETEC (est+, elt+)
32~63	EHEC/VTEC (stx1+)
64~95	EHEC/VTEC (stx2+)
96~127	EHEC/VTEC (stx1+, stx2+)
Other	Please judge based on biochemical properties etc.

Product information

Product Name	Package size	Stored at	Product No.
CicaGeneus™ Pathogenesis Gene Detection PCR Kit (for Diarrheagenic <i>E. coli</i>) 2	50 rxns	-25 °C ~ -20 °C	08377-96
CicaGeneus™ DNA Extraction Reagent	120 rxns	2 °C ~ 8 °C	08178-96

Other PCR, POT series

Product Name	Package size	Product No.	Product Name	Package size	Product No.
CicaGeneus™ Staph POT KIT	120 rxns	08180-96	CicaGeneus™ ESBL Genotype Detection KIT2	30 rxns	08116-96
	30 rxns	08180-97	CicaGeneus™ AmpC Genotype Detection KIT	30 rxns	08143-96
CicaGeneus™ Pseudo POT KIT	50 rxns	08187-96	CicaGeneus™ Carbapenemase Genotype Detection KIT2	30 rxns	08158-96
CicaGeneus™ Acineto POT KIT	30 rxns	08062-96	CicaGeneus™ staph coagulase detection set	50 rxns	08179-96
CicaGeneus™ <i>E. coli</i> POT KIT	30 rxns	08362-97	CicaGeneus™ Toxin Gene Detection KIT (for <i>C. difficile</i>)	30 rxns	08115-96
CicaGeneus™ <i>C. diff</i> POT KIT	30 rxns	08106-97			
CicaGeneus™ ECC POT KIT	30 rxns	08376-97			

*Product composition and storage temperature may change according to the country of supply.

- Please use the products listed in the catalog as reagents (chemicals used for testing or research purpose).
- Product information is subject to change without notice. For the latest information, please have a look at our website "Cica-Web".

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Please scan for more product details!

