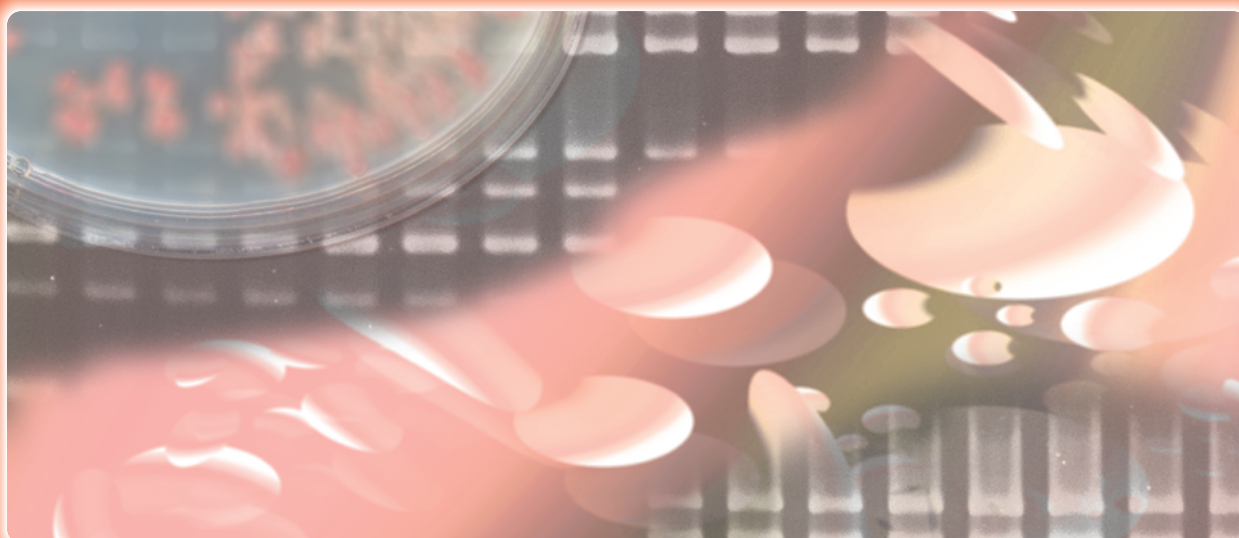


Cica Geneus[®] Acineto POT KIT



Kanto Reagents



The spread of carbapenem-resistant *Acinetobacter spp.* has become a global problem. The POT method can not only identify the species of Carbapenem-resistant *Acinetobacter calcoaceticus baumannii* (ACB) complexes but also simultaneously determine the international epidemic clone I, II and the genetic identities of *Acinetobacter baumannii* in several hours.

This POT method can easily and rapidly identify the international epidemic clones and the identities of *Acinetobacter spp.* (*A. baumannii*, *A. pittii*, *A. nosocomialis*, *Acinetobacter* genomic species close to 13TU). It is a useful tool for infection control.

※ This test method is developed by grants from the Ministry of Health, Labour, and Welfare of Japan (grants H24-Shinko-Ippan-10)

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 as Multilocus Sequence typing (MLST).
- Required for the examination time is approximately 4 hours.

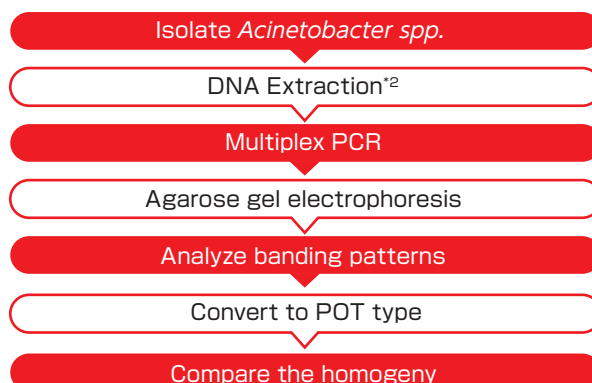
■ Composition (30 rxns)

Reagent	Volume
A AptaTaq DNA Master(5×Conc.) ^{*1}	240 μ L× 1
B PCR supplement	240 μ L× 1
C Primer mix α	120 μ L× 1
D Primer mix β	120 μ L× 1
E Positive control	240 μ L× 1
F Loading buffer(6×Conc.)	240 μ L× 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



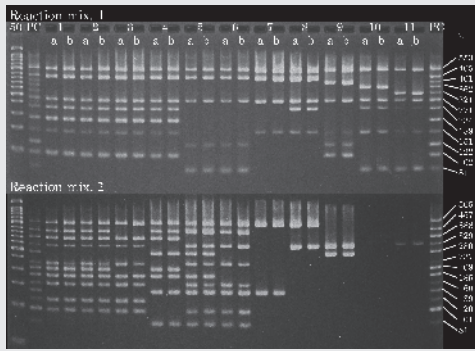


Fig.1 Example of the electrophoretic pattern

50 : 50 bp DNA Ladder, P : Positive control
 1,2 : *A. baumannii* (ST2) (Clinical isolate from the outbreaks example)
 3 : *A. baumannii* (ST2)
 4 : *A. baumannii* (ST2) Multiple-Drug-Resistant *A. baumannii*
 5 : *A. baumannii* (ST1) ATCC™ BAA1605
 6 : *A. baumannii* (ST1)
 7 : *A. baumannii* (ST152)
 8 : *A. baumannii* (ST34)
 9 : *A. pittii*
 10 : *A. nosocomialis*
 11 : *A. sp. close to A. nosocomialis*

Table.1 Kinds of detection ORF and Amplicon size

	POT No.	Amplicon size (bp)	POT modulus	Target domain
Reaction mixture 1	PCR PC	553		<i>atpA</i>
	<i>A. baumannii</i>	465		OXA-51
	<i>A. pittii</i>	401	1000	Specific gene
	<i>A. nosocomialis</i>	362	2000	Specific gene
	<i>A. sp. close to 13TU</i>	321	3000	Specific gene
	POT 1-1	271	64	Genomic Islet-1
	POT 1-2	231	32	Genomic Islet-2
	POT 1-3	189	16	Genomic Islet-3
	POT 1-4	151	8	Genomic Islet-4
	POT 1-5	122	4	Genomic Islet-5
Reaction mixture 2	POT 1-6	102	2	Genomic Islet-6
	POT 1-7	81	1	Genomic Islet-7
	PCR PC	565		OXA-51
	POT 2-1	457	32	Genomic Island-1
	POT 2-2	388	16	Genomic Island-2
	POT 2-3	329	8	Genomic Island-3
	POT 2-4	280	4	Genomic Island-4
	POT 2-5	237	2	Genomic Island-5
	POT 2-6	209	1	Genomic Island-6
	POT 3-1	185	32	Genomic Island-7
	POT 3-2	160	16	Genomic Island-8
	POT 3-3	139	8	Genomic Island-9
POT 3-4	120	4	Genomic Island-10	
POT 3-5	101	2	Genomic Island-11	
POT 3-6	81	1	Genomic Island-12	

- ① Distribution patterns of ORFs are identified by reading electrophoresis banding patterns (Fig.1) .
- ② POT types of three categories are obtained by input electrophoresis banding patterns into the Excel calculation sheet which can be downloaded from Kanto Chemical Co. Inc web site.
 - i When PCR PC is positive, the sample can confirm that it is *Acinetobacter sp.*
 - ii The value of POT 1 of *A. baumannii* become less than 1000, *A. pittii* become 1000-1999, *A. nosocomialis* become 2000-2999, *A. sp. close to 13TU* become 3000-3999, Other *A. spp.* become more than 4000.
 - iii If the value of POT 1 become 69, the strain is supposed to international clone I, If it become 122, the strain is supposed to international clone II,
 - iv You can suppose the homogeneity between strains objectively by comparing the POT type.
 - v As for the strains obtained from outbreaks, all POT types (1-3) become same. (1&2 in Fig.1)

Result

Category	Sample number in Fig.1										
	1	2	3	4	5	6	7	8	9	10	11
POT1	122	122	122	122	69	69	72	104	1105	2105	3105
POT2	59	59	26	53	46	42	0	8	0	0	0
POT3	54	54	54	41	59	27	8	0	0	0	0

Product Information

Product No.	Product Name	Package size	Stored at
08062-96	CicaGeneus® Acineto POT KIT	30 rxns	-20 °C~-25 °C
08178-96	CicaGeneus® 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
08362-97	Cica Geneus® E.coli POT KIT	30 rxns
08106-97	Cica Geneus® C. diff 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	10×TBE buffer	1 L
46509-79	10×TAE buffer	1 L
14575-43	Ethidium bromide solution	10 mL
01089-23	Agarose KANTO HC	100 g
01016-96	AptaTaq DNA Master(5×Conc.)	500 µL

- The example data of the electrophoretic pattern is provided by Dr. Arakawa of Nagoya University and Dr.Suzuki of Fujita Health University.
- This product is obtaining the patent licensing from Aichi Prefecture and Nagoya University.

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