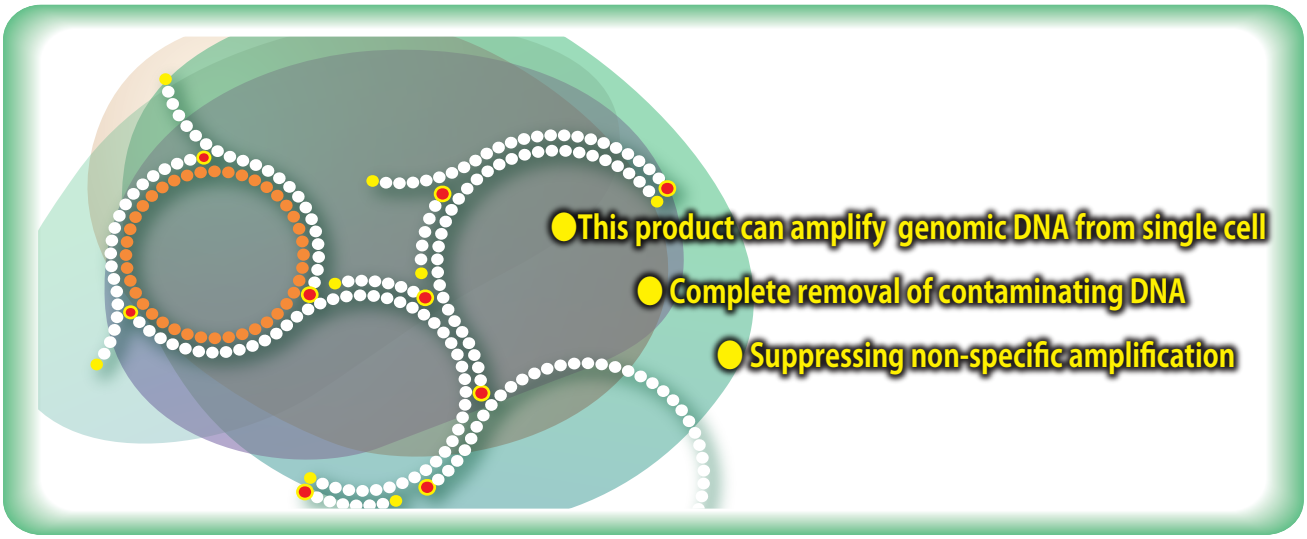
phi29 DNA Polymerase set

Kanto Reagents



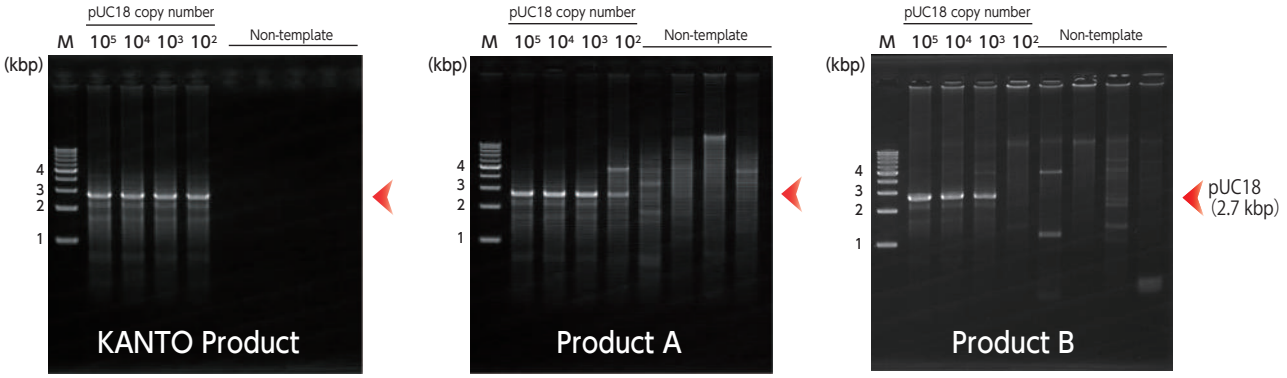
A phi29 DNA Polymerase is a replicative polymerase from *Bacillus subtilis* phi29. This polymerase has used Whole Genome Amplification (WGA) etc.

Features

- Mesophilic enzymes
- Extreme strand displacement activity
- Extreme processivity
- Extreme extensibility
- An inherent 3´→5' proofreading exonuclease activity

Example.1: Circular DNA amplification by RCA (Rolling Circle Amplification)

pUC 18 DNA was amplified with several kinds of phi29 DNA polymerase. These Amplicons treated with restriction enzyme (*Bam*H I and *Eco*R I) which were detected by agarose gel electrophoresis. (2.7 kbp)



● KANTO Product suppresses nonspecific amplification !

Product No.	Product Name	Stored at	Size
10195-96	phi29 DNA polymerase set (0.05 mg/mL)	-20～-25℃	1set (25 μL × 4)

*This product contains both phi29 DNA polymerase (0.05 mg/ mL) 25 μL×4 and 10 × Reaction Buffer 100 μL×4.
*This product has been developed through NARO(National Agriculture and Food Research Organization).

Kanto Chemical

Example.2: Human genomic amplification by MDA (Multiple Displacement Amplification)

This product used Whole Genome Amplification(WGA) by following protocol. Samples are Human genomic DNA from human blood or purified water.

Protocol

Template mix	
10 × Reaction Buffer	1.0 μL
100 μM 6R5S primer*	2.0 μL
dDW	5.0 μL
Template DNA	2.0 μL
Total	10.0 μL

↓

95℃、3 min

↓

4℃

＊ 6R5S primer : rN*rN*rN*rN*rN*rN* (6mer)[8]
(rN means to mixed base of RNA、＊ means to Phosphorothioated)

Reaction mix	
10 × Reaction Buffer	1.0 μL
25 mM dNTP	0.8 μL
PPase (1 mg/mL)	0.1 μL
dDW	5.8 μL
1M DTT	0.1 μL
100 × SYBR GreenⅡ	0.2 μL
phi29Poly (0.05 mg/mL)	2.0 μL
Total	10.0 μL

↓

Add to template mix

↓

30℃、16 hr (measuring the fluorescence every 10 min)

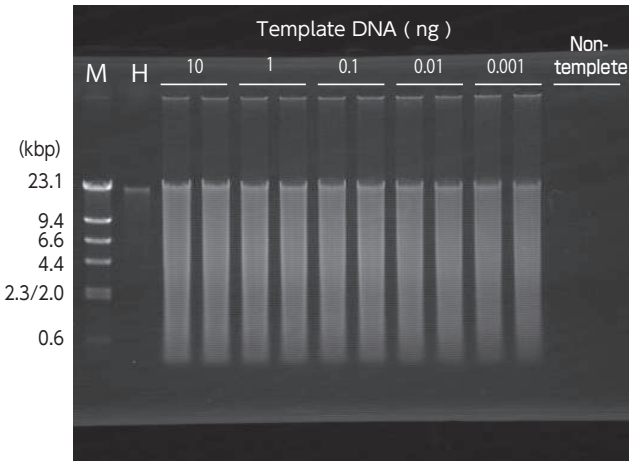
↓

65℃、10 min

↓

4℃

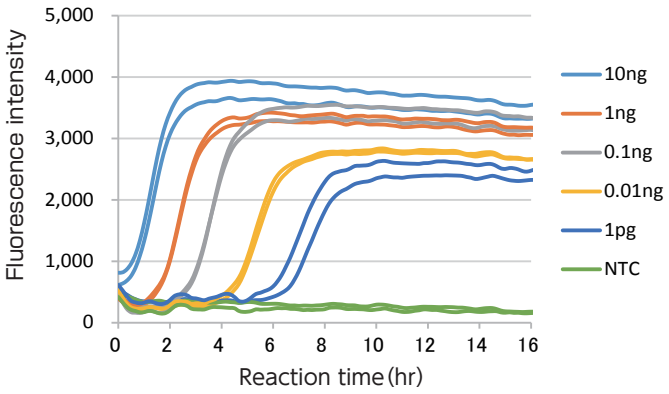
Nonspecific binding is not detected



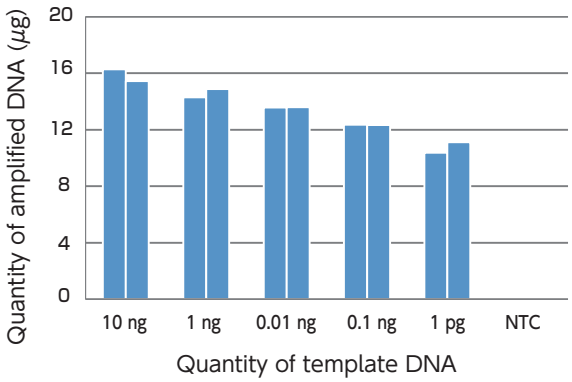
Electrophoresis banding patterns

M : λ-*Hind* Ⅲ digest marker　H : Human genomic DNA
10～0 : Amplicon after WGA 1 μL

This product can amplify DNA of 1 pg than 10 μg



Amplification plots of human genomic DNA using MDA



Quantity of amplified DNA on amplification

References

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