EZassay

Phi29 DNA polymerase v2 Instructions

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EZassay Biotechnology Ltd.

Catalog Code: PHI-250
PHI-2500

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Product Information	
Product name	Phi29 DNA polymerase v2
Expression system	Escherichia coli
Quality	Recombinant protein
Form	Liquid
Molecular weight	68kDa

Product Introduction

Phi29 DNA polymerase V2 is optimized on the basis of the original protein sequence, which enhances thermal stability, has strong strand displacement ability and processive synthesis property. Especially suitable forRolling circle amplification (RCA) and Multiple Displacement Amplification (MDA). The enzyme has 3 '→5' exonuclease activity, but no 5 '→3' exonuclease activity, which can ensure the high fidelity of amplification reaction. Therefore, it is suitable for high-fidelity isothermal PCR, and Whole Genome Amplification of single cells, pathogenic microorganisms, and metagenome. WGA), amplified DNA from dry blood spot samples, single cell genome amplification, SNP genotyping and STR/ microsatellite analysis.

Materials supplied

Cat:	PHI-250	PHI-2500
Phi29 DNA polymerase	10 U/μl *25 μl	50 U/μl *50 μl
10× Reaction Buffer	100 μl	1 ml
Diluent buffer	100 μl	1 ml

Storage

-20°C. Suggest to aliquot after receiving. Avoid repeated freeze-thaw.

Other materials required

・dNTP(10 mM)
・Random Primers（Recommend Random hexamer primers (Exo-Resistant) Cat.#: OLI-PTO-100）
・Thermal cycler or heat block

Application example

Input Material: purified genomic DNA from bacteria.
1.Prepare reactions as described in the table below.

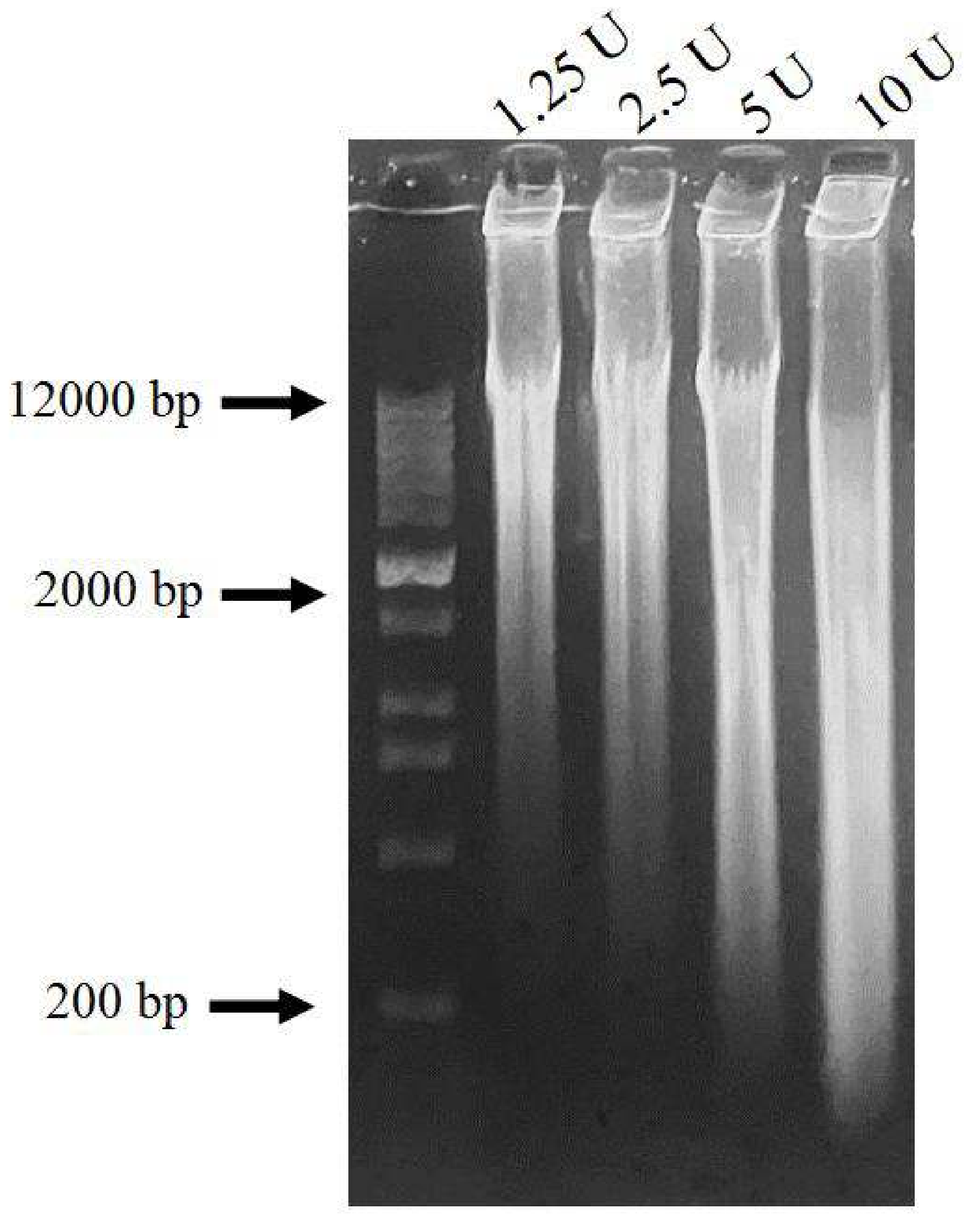
Components	Volume
Nuclease-free Water	X µl
Reaction Buffer (10X)	1 µl
Random Primers (100µM)*	1 µl
Template DNA (4ng)*	Y µl
Total Volume	8 µll

*Specific primers can be used to target specific sequence of interest.
**Template input amount can be adjusted as needed.
Incubate at 95°C for 3 min and immediately place on ice to cool for 5 min.
2.Prepare amplification as described in the table below.

Components	Volume
Product from last step	8 µl
dNTP (10mM)**	1 µl
Phi29 DNA Polymerase (5U/µl) ***	1 µl
Total Volume	10 µl

** dNTP optimization range 100~500µM；
***Phi29 DNA polymerase optimization range: 1~10 U/10µl reaction.
Vortex and briefly centrifuge. Then incubate at 30°C over-night.

Incubate at 65°C for 10 min to deactivate Phi29 DNA polymerase. The amplified product could be used for sequencing or downstream application after purification.



DNA agarose gel 0.7%, 100V, 40mins

Notes

・For DNA samples that require denaturation, incubate at 95 °C for 5 minutes, followed by 4°C or an ice bath for 2 minutes.
・For whole-genome amplification, Phi29 DNA polymerase, reaction buffer, and ddH2O can be pre-mixed with the DNA sample and incubated at 30°C for 30 minutes. The exonuclease activity of Phi29 DNA polymerase was used to remove linear DNA. Then random primers and dNTP are added to start the amplification reaction.
・A thermostatic water bath or heat block is recommended for the reaction. If using a hot-lid thermal cycler, adjust the hot-lid temperature to 40°C to avoid enzyme inactivation.
・Yeast Inorganic Pyrophosphatase (Cat.#.:PPAS-0100) can be used to further enhance the amplification of DNA.