Ultra-Pure Phi29 DNA Polymerase

Cat. No. E014

Store at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for 1 year from the date of shipping when stored and handled properly.

Description

Ultra-Pure Phi29 DNA Polymerase is a highly processive polymerase which exhibits a strong strand-displacement function. These functions **allow for highly efficient isothermal amplification of circular or linear DNA templates via rolling circle amplification (RCA), multiple displacement amplification (MDA) and/or whole genome amplification (WGA)**. Ultra-Pure Phi29 DNA Polymerase has extremely high fidelity due to its inherent 3'→5' exonuclease activity and can amplify from very small amounts of starting templates. The enzyme is subject to a rigorous multi-step purification protocol using physical, chemical, and enzymatic methods for maximum removal of contaminating genomic DNA. Quality control involves a non-specific DNase Activity Assay and qPCR DNA Contamination Test with a TaqMan probe.

Product Component	Quantity	Part No.
Ultra-Pure Phi29 DNA Polymerase	100 µl (200 rxn)	P014-1
5X Ultra-Pure Phi29 DNA Polymerase Reaction Buffer[1]	500 µl	P014-2

[1] Buffer contains 0.5 µg/µl BSA. **Keep at -20°C at all times except when using the product.**

Product Applications

- Rolling circle amplification (RCA)
- Multiple displacement amplification (MDA)
- Whole genome amplification (WGA)
- DNA template preparation for sequencing
- Protein-primed DNA amplification

Product Source

Recombinant *E. coli*.

Enzyme Storage Buffer

50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 100 mM NaCl, and 50% (v/v) Glycerol.

Protocol

1. Thoroughly thaw and mix individual components before use, and assemble reaction on ice.

Component	Volume
5X Ultra-Pure Phi29 DNA Polymerase Reaction Buffer	2 µl
dNTPs	0.4 µl
Random Primer Mix	0.5 µl
Template DNA*	Variable*
Nuclease-free ddH_2O	Up 9.5 µl

* Volume should be adjusted depending on the nature of the template: ≥ 1 pg (purified plasmid); ≥ 0.1 ul (liquid culture); ≥ 1ul colony dissolved in double distilled water (plate colony).

2. Denature the samples at 95°C for 3 minutes (see **General Notes**).
3. Cool the samples to room temperature or 4°C.
4. Add 0.5 µl Ultra-Pure Phi29 DNA Polymerase.
5. Incubate at 30°C between 4 and 16 hours (see **General Notes**).
6. Inactivate the Ultra-Pure Phi29 enzyme by incubating at 65°C for 10 minutes, and then cool to 4°C.
7. Analyze the amplification products by agarose gel electrophoresis.

General Notes

- We recommend optimizing the conditions of the 5X Ultra-Pure Phi29 DNA Polymerase Reaction Buffer to see if additional BSA is required.
- Denaturation will ensure DNA strands are well-separated, but may also result in releasing the plasmid instead of host DNA if working with plate colonies or liquid cultures.
- We recommend assembling MasterMixes and no template controls in a DNA-free environment to prevent contamination. Nuclease-free pipette tips and PCR tubes will also improve the results.
- The length of the reaction should be experimentally determined and optimized. Generally, longer templates will require extended incubation time. Therefore, we recommend performing a test reaction at several different periods (4-16 hours).

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