phi29 DNA Polymerase Kit (10 U/µL)

Product Description

phi29 DNA Polymerase exhibits strong strand displacement activity, 3'→5' exonuclease activity (high fidelity), and high processivity (>70 kb). The high processivity and strong strand displacement activity of phi29 DNA Polymerase enable efficient isothermal DNA amplification from low DNA inputs.

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Kit Contents

Kit	Kit Code	Description	Component Volume	
			25 µL kit (0.25 kU)	100 µL kit (1 kU)
phi29 DNA Polymerase Kit (10 U/µL)	**7K0104-25UL**	phi29 DNA Polymerase (10 U/µL)	25 µL	100 µL
	7K0104-100UL	10X phi29 Pol Reaction Buffer	1250 µL	1250 µL

For larger volumes, higher concentrations, and custom formats, contact the **Sales Team** at sales@watchmakergenomics.com.

Product Applications

phi29 DNA Polymerase works well in several applications including, but not limited to:

- Multiple displacement amplification (MDA)
- Rolling circle amplification (RCA)
- Whole genome amplification (WGA)
- Cell-free cloning
- Preparation of DNA template for sequencing

Unit Definition and Buffer Compositions

- One unit is defined as the amount of enzyme required to convert 50 pmol of dNTPs into a polynucleotide fraction in 10 minutes at 30°C.
- Enzyme Storage Buffer: 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, 50% Glycerol, 0.5% Tween
- 10X phi29 Pol Reaction Buffer: 330 mM Tris-acetate (pH 8.2 at 25°C), 100 mM Mg-acetate, 660 mM K-acetate, 1% (v/v) Tween 20, 10 mM DTT

Storage and Handling

phi29 DNA Polymerase Kits are shipped on ice packs. Upon receipt, store all kit components at -25°C to -15°C. Keep all components and reaction mixes on ice or a cooled reagent block during routine use. Take care to homogenize solutions thoroughly before use and during reaction setup. Do not vortex the polymerase. When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

Required Materials not Included

- Primers (see **Prior to Starting** for more detail)
- PCR-grade water
- Thermocycler

Prior to Starting

Primer design and concentration

Due to the 3'→5' exonuclease activity of phi29 DNA Polymerase, protection of the 3' ends of primers with at least two phosphorothioate internucleotide bonds is strongly recommended. Total yield will significantly decrease if 3'-modified primers are not used.

Optimal primer concentration depends on the application.

- For multiple displacement amplification, 5 µM final primer concentration is recommended.
- For rolling circle amplification, 2 to 4 µM final primer concentration is recommended.

Alkaline DNA denaturation

An alternative to thermal DNA denaturation is alkaline DNA denaturation and neutralization. 10X phi29 Pol Reaction Buffer is compatible and can be used downstream of workflows using alkaline DNA denaturation.

DTT Recommendations

DTT is required for optimal activity of phi29 and both the reaction and storage buffer supplied contain DTT to ensure this activity. However, it is recommended that for buffer stocks older than 4 months or buffers that have undergone multiple freeze/thaw cycles DTT should be replenished by adding 10 µL 1M DTT per mL of reaction buffer.

Polymerization Protocol

Note: The following example protocol is for a single reaction. When preparing multiple reactions, prepare master mixes with an appropriate overage to improve inter-sample consistency.

1. Polymerization Reaction

1.1 Thaw the 10X phi29 Pol Reaction Buffer, 10 mM dNTP Mix, and primers on ice. Vortex for 5 – 10 seconds and briefly centrifuge.

1.2 Invert the phi29 DNA Polymerase (10 U/µL) tube 10 times to mix and briefly centrifuge.

1.3 On ice, combine components as specified:

Component	Final Concentration	Volume (per 20 µL reaction)
DNA template	Variable	Variable
Modified Primers[1]	2 – 5 µM[1]	Variable
10X phi29 Pol Reaction Buffer	1X	2 µL
dNTP Mix	125 µM	0.25 µL
PCR-grade water	–	Up to 19 µL

[1]See **Prior to Starting**.

1.4 Mix thoroughly by pipetting and centrifuge briefly.

1.5 Incubate the reaction as follows to denature the DNA:

Step	Temperature (°C)	Time (min)
Lid temperature	105	N/A
DNA denaturation	94	3

1.6 Place the reaction on ice.

1.7 Add phi29 DNA Polymerase to the reaction and mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.

Component	Final Concentration	Volume (per 20 µL reaction)
Denatured DNA sample	–	19 µL
phi29 DNA Polymerase (10 U/µL)	0.5 U/µL	1 µL

1.8 Incubate the reaction as follows:

Step	Temperature (°C)	Time (min)
Isothermal Amplification	30	2 – 16 hr[1]
Heat inactivation	65	10 min

[1]Reaction time will depend on input amount and desired yield.

1.9 Proceed to the next step in your workflow.

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