# phi29 HT DNA Polymerase

# User's Instruction

## Description

phi29 HT DNA polymerase is an updated version of phi29 DNA polymerase. In addition to the strong strand displacement and continuous synthesis (> 70kb) activity of phi29 DNA polymerase, phi29 HT DNA polymerase can continuously synthesize DNA at 42°C, while the activity of phi29 DNA polymerase is very low at this temperature. In addition, phi29 HT DNA Polymerase still has a strong 3 '- 5' exonuclease proofreading function and the fidelity of the synthesized DNA fragments is high. The exonuclease activity of this enzyme is strong, so the primer needs 3'- end thio-modification in the process of synthesis to reduce the cleavage effect of the exonuclease activity on the primer.

## Kit Contents

| | 1,000U |
|---|---|
| 1. phi29 HT DNA Polymerase (10 U/μl) | 100 μl |
| 2. 10 × phi29 Buffer | 1 ml |

| | 10KU |
|---|---|
| 1. phi29 HT DNA Polymerase (10 U/μl) | 1 ml |
| 2. 10 × phi29 Buffer | 1 ml×3 |

## Features

- Strong strand displacement capability
- Continuous synthesis capability
- High fidelity of amplification

## Applications

- DNA isothermal rolling loop amplification
- DNA synthesis requiring strong strand displacement
- Rapid replication of plasmids

- Rapid replication requiring high fidelity

## Advantages

The high-temperature reaction characteristic of phi29 HT DNA Polymerase has the following advantages:

- In the next generation sequencing (NGS), the enzyme has stronger amplification activity for complex templates such as high GC content and palindrome structure, which makes the coverage of NGS more uniform and reduces the depth required for sequencing.
- High-temperature reaction conditions improve the synthesis of WGA products of genomic DNA and can be used for variable temperature amplification.
- The gap region in sequencing is reduced, which can improve the quality and integrity of the data from single-cell sequencing.
- Reduce non-specific amplification products.
- Improve the amplification performance and specificity of MDA/RCA and other experiments.

## Protocol

1. Set up the reaction as the following table:

| Component | Volume |
|---|---|
| 10×phi29 Buffer | 2 μl |
| dNTP Mixture (10 mM each) | 1.2 μl |
| Template DNA | 0.1-40 ng |
| Thio-modified Oligo | X μl |
| $ddH_2O$ | Up to 19 μl |

   - In different types of experiments, choose oligos according to the needs of the experiment.

2. Primer and template annealing: After the preparation of the system, place it in the PCR instrument, 95°C for 3min, 25°C for 3min.

3. After annealing, add 1 μl phi29 HT DNA Polymerase to the annealed product and mix

well.

4. Amplification reaction

a) Isothermal amplification: For circular DNA template, we recommend to use isothermal amplification. Incubate at 30°C for 6~16h. The enzyme can work at 30-42°C, and it can be adjusted according to the type of experiment if necessary.

b) Variable temperature amplification: For genomic DNA or cDNA templates, variable temperature amplification reaction is recommended to obtain higher yield in a short time and improve the amplification yield of low concentration templates. Make the following settings on the PCR instrument: [ 30°C for 5min, 42°C for 15s ] 72 cycles (around 6h) or 120 cycles (around 10h). If necessary, inactivation reaction can be carried out at 65°C for 10min after reaction.

**Notes**

- If necessary, the final concentration of 1 mM DTT and 0.2 mg/ml BSA can be added separately to improve the reaction efficiency.
- The optimum reaction temperature of the enzyme is 42°C (the enzyme is active at 30-42°C).
- The enzyme can be inactivated at 65°C for 10 min.
- Adjust the concentration of dNTP between 100 and 500 μM according to the needs of the experiment.
- The addition of Inorganic Yeast Pyrophosphatase (Cat. No.: IYP-20) can increase the DNA yield.
- Thio-modification of the 3' end of the reaction primer can avoid primer degradation.

## Storage

The minimum shelf life is 3 years at -20°C.

**Only for research and not intended for treatment of humans or animals**