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Phi29 DNA Polymerase

1 Packing list

Components	HY-KE8001-125U
Phi29 DNA Polymerase(10 U/μL)	12.5 μL
10× Phi29 Buffer	1 mL
100× BSA	200 μL
dNTPs (10 mM each)	200 μL

2 Introduction

Phi29 DNA Polymerase is a mesophilic DNA polymerase cloned from the Bacillus subtilis phage Phi29. It was expressed in E. coli and purified and isolated multiple times.Phi29 DNA Polymerase has special strand displacement activity and efficient continuous synthesis characteristics. It has strong binding ability to templates and can continuously synthesize DNA fragments up to 70kb without dissociating from the template.At the same time, this enzyme has a strong 3′→5′ exonuclease correction function, and its fidelity is 100 times higher than Taq DNA Polymerase.

3 Unit definition

The amount of enzyme required to incorporate 0.5 pmol of dNTPs into acid-insoluble matter within 10min at 30°C is defined as 1 unit.

4 General Protocol

Preparation of sequencing plasmid templates

1) Sample processing: Pick a single clone on the plate into 10 μL sterile water, and take 1 μL for reaction.

2) Sample pre-denaturation and plasmid amplification: According to the reaction system, pre-denaturation is performed first, and the primers and plasmids are annealed. After adding other components, amplify at 30°C overnight. Commonly used reaction systems are as follows (50 μL system):

Components	Adding amount
10× Phi29 Buffer	5 μL
Random primers (1 mM)	1.25 μL
Plasmid template (5-10 ng/μL)	1 μL
Add ddH_2O to 44.5 μL, pre-denature at 95°C for 5 min, and immediately incubate on ice for 5min.	
dNTPs (10 mM each)	2.5 μL
100× BSA	0.5 μL
Phi29 DNA Polymerase (10 U/μL)	2.5 μL

3) Amplification effect detection (optional testing): Dilute the amplification product and conduct agarose gel electrophoresis. If there is a suitable enzyme cutting site, the enzyme cutting reaction can be detected.

4) Thermal inactivation: 65℃, 10min.

5) Sequencing reaction: Take 2 μL of the above reaction solution and add 8 μL of sterile water, and it can be used for direct sequencing.

5 Storage

-20°C, 1 year

6 Precautions

1. This product is extremely sensitive, so be careful to prevent template contamination.
2. Phi29 DNA polymerase has extremely strong 3´→5´exonuclease activity and can degrade primers. Random primers modified with thio groups should be used in the amplification reaction.
3. This product is for R&D use only, not for drug, household, or other uses.
4. For your safety and health, please wear a lab coat and disposable gloves to operate.