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Bst DNA Polymerase, Large Fragment

1 Packing list

Components	HY-KE8003-800U
Bst DNA Polymerase, Large Fragment (8 U/μL)	100 μL
10× Bst LF Buffer with Mg^{2+}	2 mL×2
dNTPs (10 mM each)	200 μL

2 Introduction

Bst DNA Polymerase large fragment is a part of Bacillus stearothermophilus DNA polymerase, which is derived from E. coli strain. It is expressed in E. coli and purified and isolated multiple times. The enzyme has 5′→3′DNA polymerase activity but does not have 5′→3′exonuclease activity and can be used in DNA sequencing, DNA labeling and isothermal DNA amplification.

3 Properties

Source	E. coli
Unit definition	1 unit is defined as the amount of enzyme required to incorporate 10 nmol of dNTPs into acid-insoluble precipitate within 30min at 65°C.
10×Bst LF Buffer with Mg^{2+}	200 mM Tris-HCl, 100 mM $(NH_4)_2SO_4$, 500 mM KCl, 20 mM $MgSO_4$, 1% Tween 20, pH 8.8.
Storage buffer	50 mM KCl, 10 mM Tris-HCl (pH 7.5), 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100 , 50% Glycerol.
Reaction conditions	1× Bst LF Buffer with Mg^{2+}, 65°C warm bath.
Heat inactivation	80°C，20min

4 General Protocol

LAMP isothermal amplification

1) Take out the 10× Bst LF Buffer with Mg2+, thaw on ice, vortex for 10 sec to mix before use, and centrifuge briefly to collect.

2) Except for the template DNA, prepare the reaction mixture with the remaining components in the order shown below (reaction system is 25 μL).

Components	Adding amount
10× Bst LF Buffer	2.5 μL
F3/B3	0.2 μM (Final concentration)
FIP/BIP	0.8 μM (Final concentration)
Betaine	1 M (Final concentration)
dNTPs	400 μM (Final concentration)
M13 ssDNA template	2 μL
Bst DNA Polymerase, Large Fragment (8 U/μL)	1 μL
ddH_2O	Up to 25 μL

Note: 1. In order to prevent contamination when preparing reagents, be sure to operate in a clean workbench; 2. It is best to prepare reagents and template DNA in different areas to avoid contamination.

1) Use a pipette to gently mix and make sure there are no bubbles in the reaction system, then centrifuge to collect. Do not shake violently during the process to avoid inactivating the enzyme.

2) Use a pipette to mix evenly and centrifuge briefly to collect.

3) Incubate at a constant temperature of 60 ~ 65℃ for 1h.

4) If the experiment requires, electrophoresis analysis can be performed on a 2% agarose gel.

5 Storage

-20°C, 1 year

6 Precautions

1. Bst DNA polymerase does not have 3´→5´exonuclease activity.
2. For long-term storage, add 100 μg/mL BSA or 0.1% Triton X-100.
3. It is recommended that the reaction temperature should not exceed 70°C.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.