Bst DNA Polymerase

Introduction

Bst DNA Polymerase is a high-temperature stable DNA polymerase derived from *Bacillus stearothermophilus*, characterized by excellent thermal stability and extended elongation capabilities. This enzyme possesses 5'→3' DNA polymerase activity and strong strand-displacement activity, but lacks 5'→3' and 3'→5' exonuclease activities. This product is suitable for isothermal nucleic acid amplification reactions, such as loop-mediated isothermal amplification (LAMP), cross-primer amplification (CPA), rolling circle amplification (RCA), and other isothermal reactions based on rolling circle amplification. The isothermal amplification reactions are typically conducted at temperatures between 50-68℃, with a common temperature of 65℃.

Components and Storage

Components \ Size	K3105-8kU	Storage
***Bst* DNA Polymerase**	200 μL	-20℃
Shipping: Dry Ice	**Shelf life:** 2 years	

Protocol

1. Primer design:

For the design of loop-mediated isothermal amplification primers, please refer to http://primerexplorer.jp/e/ and version V5 is recommended. The manual can be downloaded at http://primerexplorer.jp/e/v5_manual/index.html. Refer to this manual for the preliminary screening of loop-mediated isothermal amplification primers (LAMP), and more suitable primers need to be verified by experiments.

2. Set up LAMP reactions on ice as follows:

Reagent	Volume	Final concentration
Nuclease-Free Water	(15.6-x) μL	/
10× *Bst* Reaction Buffer	2.5μL	1×
$MgSO_4$ (100 mM)	1.5μL	6 mM (8 mM total)
dNTP (25 mM each)	1.4 μL	1.4 mM each
FIP/BIP Primers (25×, 40 μM)	1 μL	1.6 μM
F3/B3 Primers (25×, 5 μM)	1 μL	0.2 μM
LoopF/B Primers (25×, 10 μM)	1 μL	0.4 μM
Template	x μL	>10 copies or more
Bst DNA Polymerase (40 U/μL)	1 μL	1600 U/mL
Total volume	25 μL	/

3. Reaction program: 65°C for 60 minutes.

4. Inactivation: 80°C for 20 minutes.

5. If necessary, examine the reaction products by 2% agarose gel electrophoresis. A positive result is indicated by the presence of gradient bands in the electrophoresis image, while a negative result is indicated by the absence of gradient bands.

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1. Unit definition: One unit is defined as the amount of enzyme that will incorporates 10 nmol of dNTP into acid insoluble material in 30 minutes at 65℃。

2. Enzyme storage buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50% Glycerol.

3. Denaturation or inactivation: *Bst* DNA Polymerase can be inactivated by heating at 80°C for 20 minutes.

注意事项

1. Isothermal amplification reaction temperature not exceed 70ºC, as higher temperatures can lead to enzyme inactivation. *Bst* DNA Polymerase is not suitable for thermal cycling sequencing or PCR.

2. To optimize the reaction, adjust the Mg^{2+} concentration (4-10 mM), the amount of enzyme (0.04-0.32 U/μL) or change the reaction temperature (50-68℃).

3. Reagent and template DNA preparation should be conducted in separate areas from where PCR product electrophoresis and other analyses are performed to avoid contamination.

4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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