EZassay

Hot-start Bst 2.0 DNA polymerase Instructions

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Catalog Code: BST2-1600
BST2-8000

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Product Information	
Product name	Hot-start Bst 2.0 DNA polymerase
Expression system	Escherichia coli
Quality	Recombinant protein
Form	Liquid

Product Introduction

Hot-start Bst 2.0 DNA polymerase is proprietary mutant of Bst DNA polymerase, large fragment, with a fast reaction speed and increased sensitivity and tolerance to inhibitors. It contains aptamer that ensures the enzyme is inactivated by over 90% when below 30°C. Within 1 minute after the temperature exceeds 60°C, the enzyme activity is fully restored. This feature allows the reaction system to be set up at room temperature and significantly reduces non-specific amplification under low-temperature conditions.

Hot-start Bst 2.0 DNA polymerase contains 5´→3´ DNA polymerase activity and strong strand displacement activity but lacks 5´→3´ exonuclease activity. Bst 2.0 DNA Polymerase enables highly efficient loop-mediated isothermal amplification (LAMP).

Storage

-20°C. Suggest to aliquot after receiving. Avoid repeated freeze-thaw.

Materials supplied

Cat:	BST2 -1600	BST2 -8000
Hot-start Bst 2.0 DNA polymerase (8 U/μl)	1600U	8000U
Isothermal Amplification Buffer (10X)	0.5mL	0.5mL*6
MgSO4 (100mM)	0.5mL	0.5mL*4

Unit definition

One unit is defined as the amount of enzyme that will incorporate 25 nmol of dNTP into acid insoluble material in 30 minutes at 65°C.

Inactivation

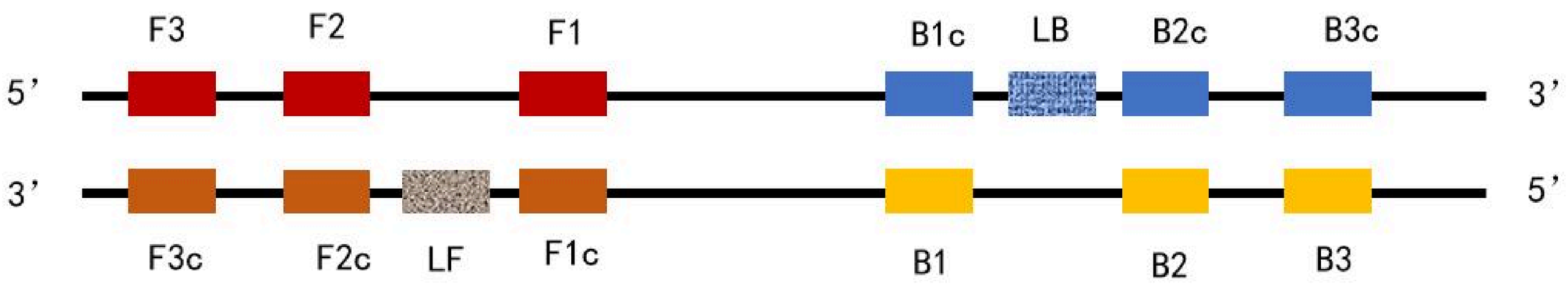
85°C for 10 minutes.

Enzyme storage solution

50 mM Tris-HCl，pH 8.0，50 mM KCl，0.1% Tween-20，0.1% Triton X-100，1 mM DTT，50% Glycerol

Other materials required

i. Template DNA or RNA
ii. SYBR™ or Eva Green™ dye or Probes
iii. dNTP Mix
iv. Nuclease-free water
v. Q-PCR thermal cycler or heat block
vi. Primers (Recommend LAMP primer online design software: https://lamp.neb.com/#!/)



- F1c: Complementary sequence of F1
- B1c: Complementary sequence of B1
- FIP: Forward Inner Primer (F1c + F2)
- BIP: Backward Inner Primer (B1c + B2)
- LF: Forward loop primer
- LB: Backward loop primer

Recommended reaction system

1、 Prepare reactions as following table. Reactions should be setup on ice.

Component	Volume	Working concentration
Isothermal Amplification Buffer (10X)	2.5 µl	1X
MgSO4 (100mM)	1.5 µL	6mM+2mM in buffer=8mM final
dNTPs (10mM)	3.5ul	1.4mM
Primers(10X)*	2.5 µl	1.6 µM FIP/BIP, 0.4 µM LF/LB, 0.2 µM F3/B3,
Hot-Start Bst2.0 (8 U/µL)	1 µl	0.32 U/µL
DNA sample	1 µl	>10 copies or more
ddH2O	Up to25 µl	-

*Sample pipetting example (preparation of 10X Primers Mix)：1、Stock primer concentrations: Dilute FIP and BIP primers to 80µM ;dilute LF, LB, F3 and B3 primers to 20 µM.；Pipetting volumes: 0.5 µL each for FIP, BIP, LF and LB primers; 0.25 µL each for F3 and B3 primers. (The primers may also be diluted to other concentrations. In such cases, the added volumes shall be calculated accordingly based on the required final concentrations in the reaction system.)

2、Incubate isothermally at 65 °C for 1 h, followed by heating at 85 °C for 10 min. The products can then be analyzed by electrophoresis on a 2% agarose gel. If required, fluorescent dye may be added to the reaction system. Fluorescence signals can be read every minute using a real-time fluorescence quantitative PCR instrument.

Notes

・Designate and use distinct areas for sample preparation, reaction setup and analysis to avoid carry-over contamination.
・Running a negative control is strongly recommended to ensure amplification specificity.
・To achieve good reproducibility, add the template last.
・If PCR thermal cycler is used, turn off the lid heating function or set it to 70 °C.
・Non-specific products are common in isothermal amplifications including LAMP. AapCas12b protein (Cat.: CAS-12P-010) or BrCas12b protein (Cat.: CAS-12R-010) or RNaseH2 enzyme (Cat.: RH2-250) can be used to increase the specificity.