Bst DNA Polymerase

User's Instruction

Description

Bst DNA Polymerase is a homologous protein of Bst DNA Polymerase, large fragment, derived from the thermophilic bacterium Bacillus stearothermophilus (Bst). When compared to the large fragment of Bst DNA Polymerase, it exhibits stronger 5'→3' DNA polymerase activity, enhanced strand displacement capability, tolerance to dUTP, salt resistance, and resistance to non-ionic detergents. Bst DNA Polymerase lacks 5'→3' and 3'→5' exonuclease activity. It can be used in various applications, including loop-mediated isothermal amplification (LAMP), crossing priming amplification (CPA), rolling-circle amplification (RCA), and isothermal amplification reactions based on rolling-circle amplification.

The isothermal amplification temperature mediated by Bst DNA Polymerase generally falls between 50-68°C, typically at 65°C. The optimal temperature depends on the primers and the products being amplified and may require experimental optimization.

Compared to the **Bst 2.0 DNA Polymerase** from similar companies, this product exhibits **similar enzyme activity, comparable high dUTP tolerance, similar high tolerance to non-ionic detergents, and equivalent salt resistance.**

Kit Contents

	40KU
1. Bst DNA Polymerase (40U/μl)	1 ml
2. 10 × Isothermal Buffer	3 ml
3. 100 mM $MgSO_4$	2 ml

Protocol

1. Set up isothermal amplification reaction as the following table:

Component	Volume	
Nuclease-free Water	(15.6-x)μl	-

10 × Isothermal Buffer	2.5μl	1X
$MgSO_4$ (100mM)	1.5μl	6mM (8mM total)
dNTP (25mM each)	1.4μl	1.4mM each
FIP/BIP Primers (25X, 40μM)	1μl	1.6μM
F3/B3 Primers (25X, 5μM)	1μl	0.2μM
Loop F/B Primers (25X, 10μM)	1μl	0.4μM
Template	xμl	> 10 copies or more
Bst DNA Polymerase (40U/μl)	1μl	1600U/ml
Total volume	25μl	-

Note

- After the completion of the reaction system setup, add an appropriate amount (1μl) of high-concentration SYBR Green I to the reaction tube cap for every 25μl of the reaction system. After the isothermal amplification reaction is finished, centrifuge at 8000×g for 1 minute. A positive reaction is indicated by the fluorescence turning green, while a negative reaction is indicated by the reaction system remaining colorless or brown. Alternatively, an indicator may not be added; a positive reaction can be recognized by the reaction solution becoming visibly turbid after the reaction program, whereas a negative reaction is indicated by the reaction solution remaining clear.
- For reaction optimization, adjust the Mg^{2+} concentration (4-10mM), enzyme amount (0.04-0.32U/μl), or change the reaction temperature (50-68ºC).
- If analysis is conducted through agarose gel electrophoresis or any method requiring the opening of the LAMP reaction container, establish separate areas and equipment for auxiliary analysis to prevent contamination.
- Due to the rapid nature of the reaction, to ensure reproducibility of the experiment, it is recommended to add the template DNA at the final stage.
- It is strongly recommended to include a negative control without the template to ensure the specificity of the amplification.
- To prevent contamination during reagent preparation, it is essential to operate within a clean bench.
- The preparation of reagents and template DNA should preferably be conducted in different areas from the analysis of PCR products by techniques such as gel electrophoresis to avoid contamination.

2. Thermo Condition:

   a) 65°C for 60 min

b) 85°C for 20 min (inactivation)

Storage

Store at -20°C. Avoid multiple freeze-thaw cycles.

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