Bst II Pro DNA Polymerase Large Fragment (Glycerol-free)

PL703

Version 23.1

Product Description

Bst II Pro DNA Polymerase Large Fragment (Glycerol-free) is obtained from the large fragment of *Bacillus stearothermophilus* DNA Polymerase by directed genetic engineering. It contains the 5'→3' polymerase activity and strong strand displacement activity, but lacks 5'→3' exonuclease activity. It is applicable for isothermal amplifications such as LAMP (Loop-Mediated Isothermal Amplification), HDA (Helicase-Dependent Amplification) and RCA (Rolling Circle Amplification). Bst II Pro DNA Polymerase Large Fragment (Glycerol-free) combines a new generation of hot start technology to inhibit polymerase activity at temperatures below 50°C and release polymerase activity at temperatures above 50°C. It displays good amplification speed, high specificity, strong salt tolerance and reliable thermal stability. The reaction system can be prepared at room temperature. This product is a glycerin-free formula, which can be used to develop lyophilized products. It should be noted that excipient ingredients are not included, please add it according to actual requirements.

Components

Components	PL703-01 (1,600 U)	PL703-02 (8,000 U)
Bst II Pro DNA Polymerase Large Fragment (Glycerol-free, 8 U/μl)	200 μl	1 ml
10 × IsothermalAmp Buffer (Freeze-dryable)	500 μl	3 × 1 ml
$MgSO_4$ (100 mM)	300 μl	2 × 1 ml

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

It is applicable for various isothermal amplification reactions such as LAMP, HDA, and RCA.

Source

Bst II Pro DNA Polymerase Large Fragment comes from *Bacillus stearothermophilus*.

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble material in 30 min at 65°C.

Self-prepared Materials

Reagents: dNTP Mix, FIP/BIP Primers, F3/B3 Primers, LoopF/LoopB Primers, Nuclease-free ddH_2O.
Instruments: qPCR instrument, PCR instrument or water bath.

Notes

For research use only. Not for use in diagnostic procedures.

1. It is not applicable for PCR.
2. Control the reaction temperature ≤70°C.

Experiment Process

Take LAMP as an example:

1. Thaw 10 × IsothermalAmp Buffer on ice. Vortex for 10 sec to mix thoroughly before use, then centrifuge briefly to the bottom of the tube.

2. Follow the table below to prepare the reaction system. The template should be added in the last step.

Components	Volume	Final Concentration	
10 × IsothermalAmp Buffer (Freeze-dryable)	2.5 μl	1 ×	
$MgSO_4$ (100 mM)	1.5 μl	6 mM (total 8 mM)	
dNTP Mix (10 mM each)	3.5 μl	1.4 mM each	
FIP/BIP Primers (100 μM)	0.4 μl each	1.6 μM each	
F3/B3 Primers (100 μM)	0.05 μl each	0.2 μM each	
LoopF/LoopB Primers (100 μM)	0.2 μl each	0.8 μM each	
Bst II Pro DNA Polymerase Large Fragment (Glycerol-free, 8 U/μl)	1.0 μl	0.32 U/μl	
DNA Template	1.0 - 5.0 μl		
Nuclease-free ddH_2O	up to 25 μl		

▲ 10 × IsothermalAmp Buffer (Freeze-dryable) may have a small amount of solid precipitated when it is just thawed, please mix well before use.

▲ The concentration of Mg^{2+} can be adjusted between 6 - 10 mM.

▲ If the experiments requires an anti-contamination system, it is recommended that add dUTP (Vazyme #P033) to a final concentration of 1.4 mM, and UDG enzyme to a final concentration of 0.04 U/μl.

▲ If the amount of primers is small, it is recommended to premix the primers first.

▲ It is recommended to prepare reagents and templates in different areas to avoid contamination.

3. Vortex to mix thoroughly, then centrifuge briefly to the bottom of the tube.

▲ Make sure there are no air bubbles in the reaction system.

4. Add template DNA. The final volume of the reaction system should be 25 μl.

▲ It is recommended to add the template last to ensure the reliability of the results, because the amplification reaction will start immediately once the template is added.

5. Vortex to mix thoroughly, then centrifuge briefly to the bottom of the tube.

6. Incubate at 60 ~ 65°C for 30 - 60 min.

FAQ & Troubleshooting

◇ How to design and screen primers for loop-mediated isothermal amplification?

Please refer to http://primerexplorer.jp/e/ for primer design. Version 5 is recommended.

Log in to http://primerexplorer.jp/lampv5e/index.html to download the manual.

For preliminary screening, please refer to the manual. The optimal primer need to be verified by experiments.

◇ How to detect the amplification product?

Both dye-based method and probe-based method can be used to detect amplification products.