Bst II DNA Polymerase Large Fragment

P702

Version 23.1

Product Description

Bst II DNA Polymerase Large Fragment 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). Compared with the previous generation, Bst II DNA Polymerase Large Fragment has superior amplification speed, dUTP tolerance, salt tolerance and thermal stability.

Components

Components	P702-01 (1,600 U)
Bst II DNA Polymerase Large Fragment (8 U/μl)[a]	200 μl
10 × IsothermalAmp Buffer	500 μl
$MgSO_4$ (100 mM)	300 μl

a. It contains 10 mM Tris-HCl pH 7.5, 50 mM KCl, 0.1 mM EDTA, 0.1% Triton X-100 and 50% Glycerol.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It is applicable for isothermal amplifications 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℃.

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℃.

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	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 DNA Polymerase Large Fragment (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	

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

▲It is recommended to premix the primer before preparing the reaction system due to its small amount.

▲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℃ for 30 - 60 min.

FAQ & Troubleshooting

1. Primer design guidance and screening 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.

2. Amplification products detection

Both dye-based (e.g. SYBR Green) method and probe-based method can be used to detect amplification products.