Bst DNA Polymerase, Exonuclease Minus

For research use only

Cat. No./Spec.

Cat. No	P1111	P1112	P1113	P1114
Spec.	1,000U	2,000U	8,000U	40,000U

Description

Bst DNA Polymerase, Exonuclease Minus is derived from Bacillus stearothermophilus DNA Polymerase I. This enzyme possesses 5'-3' polymerase activity, strand displacement activity, and dUTP tolerance, while the 5'-3' exonuclease activity has been removed. This product is suitable for contamination-resistant DNA isothermal amplification reactions, such as LAMP (loop-mediated isothermal amplification), SDA (cross-priming amplification), and other isothermal amplification techniques.

Components

Component	**P1111**	**P1112**	**P1113**	**P1114**
Bst DNA Polymerase, Exonuclease Minus	25 μl	50 μl	200 μl	1 ml
10X DNA Polymerase Buffer B	125 μl	250 μl	1 ml	1 ml × 3
$MgSO_4$ (100 mM)	125 μl	250 μl	1 ml	1 ml × 3

Unit Definition

One unit catalyzes the incorporation of 10 nmol of dNTP into acid-insoluble material in 30 minutes at 65°C.

Storage

Store at -20°C for 2 years.

Storage Buffer of Bst: 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, and 50% Glycerol.

1X Buffer B: 20 mM Tris-HCl pH 8.8, 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 2 mM $MgSO_4$, 0.1 % Tween-20。

Application

LAMP, CPA, RCA, and other isothermal amplification reactions.

Inactivation Condition

85°C for 5 minutes.

DNA LAMP Protocol

1. Prepare the reaction system

Component	**25-μl rxn**	**Final Conc.**
10X DNA Polymerase Buffer B [1]	2.5 μl	1X
$MgSO_4$ (100 mM) [2]	0.75 μl	3 mM (total 5 mM)
dNTPs Mix (10 mM) [3]	3.5 μl	1.4 mM each
[optional] dUTP (10 mM) [3]	3.5 μl	1.4 mM each
[optional] UDGase (1 U/μl) [3]	1 μl	0.04 U/μl
10X Primers [4]	2.5 μl	-
Bst DNA Polymerase, Exonuclease Minus	1 μl	1.6 U/μl
DNA sample	10ng~1μg	10 copies or more
Nuclease-free Water	to 25 μl	-

[1] 1X Buffer B contains 2 mM $MgSO_4$.

[2] Recommended concentration of Mg^{2+}: 4~10 mM.

[3] Products can be ordered separately. dNTPs, #P9013, dUTP, #P9111, Heat-Labile UDG, #R5001/R5002.

[4] 10X Primers contain 16 μM FIP/BIP, 2 μM F3/B3, 4 μM Loop F/B.

2. Reaction system

Temperature	**Time**
25~37°C (optional, to degrade template containing U)	5~10 min
65°C	30~60 min
85°C	5 min

Workflow:

In order to minimize cross-contamination, steps 6 onward should be done in an area separate from the area where you are preparing the reaction mix.

1. Thaw all kit components and hold on ice.

2. All components should be mixed well before use. Vortex all tubes for 10 seconds, then centrifuge briefly to collect.

3. Prepare the reaction mix as shown in Table 1 in the order listed. Add all the components except the target. During this step the reaction mix tube should always be held on the ice to prevent the background activity of the enzyme.

4. After all reagents have been added, mix the reaction completely. Gently vortex. This step is required to ensure uniform distribution of all reaction components.

5. Dispense reaction mix (25 μL minus sample volume) into a PCR tube or 96-well PCR plate well for each reaction.

6. Add volume of target to each well/tube for a total volume of 25 μL per well.

7. Run a no-template control (negative control) to ensure amplification specificity.

8. Cap tubes or seal plate wells. Centrifuge briefly to collect prior to incubation.

9. Incubate reactions at desired temperature for 30-60 minutes. Running a temperature gradient from (55–65 °C) is strongly recommended to determine optimum temperature.

10. If required, run samples on a 2% agarose gel.

11. If optimization is required, try adjusting the Mg^{2+} or Bst DNA polymerase concentration.

Note: Reactions may be kept at -20 °C for longer term storage.