Cat.:E105

AcuGenix™ Bst Hot Start DNA Polymerase-V3

V01

Product Description

Bst DNA polymerase is derived from Bacillus stearothermophilus DNA Polymerase I. Bst DNA polymerase retains the activity of 5'→3' DNA polymerase by genetic engineering technology, removes the activity of 5'→3'exonuclease, and has the ability of strand displacement. It can be used for DNA strand displacement reaction and LAMP (Loop-mediated isothermal amplification) amplification. The hot-start thermostatic amplification enzyme obtained by reversible modification technology based on the genetically modified AcuGenix™ Bst Hot Start DNA Polymerase-V3 can completely block the activity of the enzyme at room temperature. Therefore, the reaction can be established at room temperature to prevent non-specific amplification and improve the reaction efficiency. In addition, AcuGenix™ Bst Hot Start DNA Polymerase-V3 does not require a separate activation step. AcuGenix™ Bst Hot Start DNA Polymerase-V3 is a new generation of Bst enzyme with high sensitivity, high sample compatibility, and certain tolerance to dUTP.

Size

8 U/μL

Components

1. 8 U/μL AcuGenix™ Bst Hot Start DNA Polymerase-V3
2. 10×LAMP Buffer (Mg^{2+} free) (optional)
3. 35.71× $MgSO_4$ (optional)

*The 10×LAMP Buffer (Mg^{2+} free) does not contain dNTPs or Mg^{2+}. When preparing the reaction system, dNTPs and $MgSO_4$ must be added.

Unit Definition

One activity unit (U) refers to the amount of enzymes required to incorporate 10 nmol of deoxynucleotides into acid-insoluble substance using activated salmon sperm DNA as a template/primer at 65℃ for 30 min.

Thermal inactivation

85℃，5 min or 95℃，2 min.

Storage

Store at -20±5℃. Vortex before use. Avoid freeze-thaw cycles.

Application

1. DNA strand replacement reaction.
2. LAMP isothermal amplification.
3. DNA sequencing rich in GC templates and nanogram templates.

Prepare Reaction Mix

Component	Volume per Reaction	Concentration in Master Mix
10×LAMP Buffer (Mg^{2+} free)[1]	5 μL	1×
dNTPs（25 mM each dNTP）	2.8~3.2 μL	1.4~1.6 mM
35.71× $MgSO_4$	1.2~1.6 μL	1×
8 U/μL AcuGenix™ Bst Hot Start DNA Polymerase-V3	2 μL	16 U
50×Eva Green[2]	1 μL	1×
10×Primer Mix[3]	5 μL	1×
Template[4]	—	—
ddH_2O	To 50 μL	—

1. The 10×LAMP Buffer (Mg^{2+} free) does not contain dNTPs or Mg^{2+}. When preparing the reaction system, dNTPs and $MgSO_4$ must be added.
2. The 50× Eva Green can be replaced with other dyes, and the dosages of different dyes should be adjusted in accordance with experimental requirements.
3. 10×Primer Mix: 16 μM FIP, 16 μM BIP, 2 μM F3, 2 μM B3, 4 μM LoopF, and 4 μM LoopB in TE buffer.
4. For optimal amplification, add an appropriate amount of template: plasmid DNA 10 fg-1 ng, genomic DNA 1 ng-100 ng, or appropriately prepared DNA extract.

Zhuhai Biori Biotechnology Co.,Ltd
Tel: +86-0756-8699969 Web: www.biori-en.com Email: marketing@biori-en.com
Addr:No. 88, Shui' an 1st Road, Xiangzhou District, Zhuhai, Guangdong 519060, China

Reaction Conditions

60~65℃, 30~60 minutes (Collect fluorescence per minute).

Q & A

LAMP amplification products are scarce or non-existent:

1. Elevate the quantity of the template and the concentration of the enzyme.
2. Substitute the primers. It is advisable to design multiple primer sets and conduct screening for the optimal primers via preliminary experiments. For primer design, refer to http://primerexplorer.jp/e/.
3. The use of Loop Primers is recommended to enhance amplification efficiency and reduce amplification time.