

Product Description

AcuGenix™ Bst DNA Polymerase-V3 is derived from *Bacillus stearothermophilus* DNA Polymerase I, and its 5'→3' DNA polymerase activity has been retained through genetic engineering, while its 5'→3' exonuclease activity has been removed. It has the ability to replace DNA strands, making it suitable for strand displacement reactions and LAMP (Loop-mediated isothermal amplification) amplification. AcuGenix™ Bst DNA Polymerase-V3 has been genetically modified to improve its sensitivity, and compared to the wild-type Bst DNA polymerase and long fragments, this enzyme can effectively increase the amplification speed and yield.

Size

8 U/μL

Components

1. 8 U/μL AcuGenix™ Bst DNA Polymerase-V3
2. 10×LAMP Buffer (Mg²⁺ free) (optional)
3. 35.71×MgSO₄ (optional)

*The 10×LAMP Buffer (Mg²⁺ free) does not contain dNTPs or Mg²⁺. When preparing the reaction system, dNTPs and MgSO₄ must be added.

Unit Definition

One enzyme unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol dNTP into a 65°C acid-insoluble material in 30 minutes.

Thermal inactivation

85°C, 5 min or 95°C, 2 min.

Storage

Store at -20±5°C. 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 DNA Polymerase-V3	2 μL	16 U
50×Eva Green ²	1 μL	1×
10×Primer Mix ³	5 μL	1×
Template ⁴	—	—
ddH ₂ O	To 50 μL	—

1. The 10×LAMP Buffer (Mg²⁺ free) does not contain dNTPs or Mg²⁺. When preparing the reaction system, dNTPs and MgSO₄ 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.

Reaction Conditions

60~65°C, 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.