Cat.: FE103

AcuGenix™ Bst Hot Start DNA Polymerase (Glycerol-Free)-V2

V01

Product Description

Bst DNA polymerase is derived from *Bacillus stearothermophilus* DNA Polymerase I. Through genetic engineering techniques, it retains the 5'→3' DNA polymerase activity while eliminating the 5'→3' exonuclease activity and exhibits strand displacement capability. It is suitable for DNA strand displacement reactions and LAMP (Loop-mediated isothermal amplification). AcuGenix™ Bst Hot Start DNA Polymerase (Glycerol-Free)-V2 is a specialized enzyme optimized and formulated according to the characteristics of lyophilized reagents. It is a thermosensitive isothermal amplification enzyme obtained via reversible modification technology based on genetically engineered Bst 2.0 DNA polymerase. The activity of this enzyme can be completely inactivated at room temperature, allowing reactions to be initiated under ambient conditions. This suppresses non-specific amplification and improves reaction efficiency. Furthermore, AcuGenix™ Bst Hot Start DNA Polymerase (Glycerol-Free)-V2 does not require separate activation steps.

Size

8 U/μL

Components

1. 8 U/μL AcuGenix™ Bst Hot Start DNA Polymerase (Glycerol-Free)-V2
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 unit is defined as the quantity of enzyme that integrates 10 nmol of dNTP into acid-insoluble substances within 30 minutes at a temperature of 65℃.

Thermal inactivation

This product undergoes thermal inactivation at 85℃ for 5 minutes or 95℃ for 2 minutes.

Storage

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

Application

This product is suitable for DNA strand displacement reactions and LAMP (Loop - mediated isothermal amplification).

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Concentration
8 U/μLAcuGenix™ Bst Hot Start DNA Polymerase (Glycerol-Free)-V2	1 μL	2 μL	0.32 U/μL
10×LAMP Buffer (Mg^{2+} free) [1]	2.5 μL	5 μL	1×
dNTPs (25 mM each dNTP)	1.4-1.6 μL	2.8-3.2 μL	1.4-1.6 mM
250 mM $MgSO_4$	0.6-0.8 μL	1.2-1.6 μL	6-8 mM
50×Eva Green [2]	0.5 μL	1 μL	1×
10×Primer Mix [3]	2.5 μL	5 μL	1×
Template DNA [4]	——	——	——
ddH_2O	To 25 μL	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. 50×Eva Green can be substituted with other dyes. The optimal amount may vary depending on the dye used and needs to be determined experimentally.
3. Recommended primer concentration: The 10× Primer Mix comprises 16 μM FIP, 16 μM BIP, 2 μM F3, 2 μM B3, 4 μM LoopF, and 4 μM LoopB. Concentration gradient testing can be carried out to determine the optimal primer dosage. It is recommended that primers be prepared with 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.

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Reaction Conditions

Incubate at a temperature range of 60-65℃ for a duration of 30-60 minutes, and acquire fluorescence data at one-minute intervals. After the reaction is completed, allow the sample to cool to ambient temperature before analyzing the results.

Quality control

1. Functional testing: sensitivity, specificity, reproducibility of LAMP.
2. No exogenous nuclease activity, no exogenous endo/exonuclease contamination.

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.