AcuGenix™ Bst Hot Start DNA Polymerase-V2

Product Description

AcuGenix™ Bst Hot Start DNA Polymerase-V2 is derived from Bacillus stearothermophilus DNA Polymerase I. AcuGenix™ Bst Hot Start DNA Polymerase-V2 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 Bst DNA polymerase-Series Ⅱ 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-V2 does not require a separate activation step.

Size

8 U/μL

Components

1. 8 U/μL AcuGenix™ Bst Hot Start DNA Polymerase-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 enzyme unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol dNTP into a 65℃ acid-insoluble material in 30 minutes.

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

Components	25 μL System	50 μL System	Final Concentrature
AcuGenix™ Bst Hot Start DNA Polymerase-V2 (8 U/μL)	1 μL	2 μL	0.32 U/μL
10×LAMP Buffer	2.5 μL	5 μL	1×
250mM $MgSO_4$	0.6-0.8 μL	1.2-1.6 μL	1×
25mM dNTP Mix	1.4-1.6 μL	2.8-3.2 μL	1×
10×Primer Mix [1]	2.5 μL	5 μL	1×
50×Eva Green [2]	0.5 μL	1 μL	1×
Template DNA [3]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. 10×Primer Mix: 16μM FIP, 16μM BIP, 2μM F3, 2μM B3, 4μM LoopF and 4μM LoopB in TE Buffer.
2. 50×Eva Green can be replaced by other dyes, and the dosage of different dyes needs to be adjusted according to the experiment.
3. The gradient dilution experiments of sample is helpful to determine the optimal amount of target.

Reaction Conditions

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

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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.