vivantis

DNA AMPLIFICATION PRODUCT

Product Datasheet

HS Bst DNA Polymerase, Large Fragment, Hot Start

Product No	: PL6203
Quantity	: 800U

Bst

Lot :
Expiry Date :
Concentration : 8U/μL
Supplied with : 500 μL of 10X HS Bst IsoAmp Buffer
300 μL of 100 mM $MgSO_4$

Store at - 20 °C

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Description:

HS Bst DNA Polymerase is the recombinant DNA polymerase enzyme obtained from the large fragment of *Bacillus stearothermophilus* DNA Polymerase by directed genetic engineering. It contains the 5'→3' DNA polymerase activity and strong strand displacement activity, but lacks 5'→3' exonuclease activity. HS Bst DNA Polymerase combines a new generation of **HOT START** technology to inhibit polymerase activity at temperatures below 50°C and release polymerase activity at temperatures above 50°C. The reaction system can be prepared at room temperature. Compared with wild-type, HS Bst DNA Polymerase with Hot Start is suitable for isothermal amplification, featuring high amplification ability with superior amplification speed, high specificity and thermal stability. This product have supplied isothermal reaction buffer; suitable to be used for isothermal DNA amplification with different tests designs.

Unit Definition :

1 unit (U) is defined as the amount of the enzyme that is required to incorporate 10 nmol of dNTPs into an acid-insoluble material in 30 minutes at 65°C.

Storage Buffer :

10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 50% (v/v) glycerol, 0.1% Triton®X-100.

Reaction Buffer:

10X HS Bst IsoAmp Buffer*:

100 mM $(NH4)_2SO_4$, 500 mM KCl, 20 mM $MgSO_4$, 1% Tween®20.
* This buffer is pH-sensitive.
* Prepare small aliquot of buffer before use to avoid repeated freeze thaw cycles.

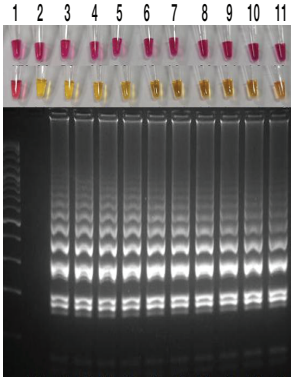
100 mM $MgSO_4$

Application:

Isothermal amplification (LAMP), HDA (Helicase-Dependent Amplification) and RCA (Rolling Circle Amplification) and related research.

Quality Control:

All preparations are assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.



Amplification using HS Bst DNA Polymerase, Large Fragment

Lane 1: 100 bp Plus Ladder
Lane 2 (Tube 1): NTC (8.0U HS Bst DNA Polymerase)
Lane 3 (Tube 2): 8.0U HS Bst DNA Polymerase
Lane 4 (Tube 3): 7.2U HS Bst DNA Polymerase
Lane 5 (Tube 4): 6.4U HS Bst DNA Polymerase
Lane 6 (Tube 5): 5.6U HS Bst DNA Polymerase
Lane 7 (Tube 6): 4.8U HS Bst DNA Polymerase
Lane 8 (Tube 7): 4.0U HS Bst DNA Polymerase
Lane 9 (Tube 8): 3.6U HS Bst DNA Polymerase
Lane 10 (Tube 9): 2.4U HS Bst DNA Polymerase
Lane 11 (Tube 10): 1.6U HS Bst DNA Polymerase
Lane 12 (Tube 11): 1.0U HS Bst DNA Polymerase

2% TBE agarose pre-stained gel, 5V/cm

Recommended Protocol for LAMP Test using HS Bst DNA Polymerase:

A. Preparation of reaction buffer

1. Thaw 10X HS Bst IsoAmp Buffer on ice. Vortex for 10 seconds to mix thoroughly before, then centrifuge briefly to the bottom of the tube.
2. Follow the table below to prepare the reaction system. The template should be added in the last step.

Reagent :	Volume	Final Concentration
10X HS Bst IsoAmp Buffer	2.5 μL	1X
100 mM $MgSO_4$	1.5 μL	6 mM
10 mM dNTP Mix	3.5 μL	1.4 mM
10 μM FIP[a] / BIP[a] Primer	4.0 μL each	1.6 μM
10 μM F3[a] / B3[a] Primer	0.5 μL each	0.2 μM
10 μM LF[a] / LB[a] Primer	1.0 μL each	0.4 μM
8U/μL HS Bst DNA Polymerase	1.0 μL	0.32U
25mM Phenol Red, pH 8.2 using 1M KOH	0.1 μL	0.1 mM
Template[b]	1.0 μL	Variable
Nuclease-free water	Adjust final volume to 25 μL	

[a] It is recommended to premix the primer before preparing the reaction system due to its small amount.
[b] It is recommended to prepare reagents and templates in different areas to avoid contamination.

3. Vortex to mix thoroughly, then centrifuge briefly to the bottom of the tube.*
* Make sure there are no air bubbles in the reaction system.
4. Add template DNA. The final volume of the reaction system should be 25 μL.*
* It is recommended to add the template last to ensure the reliability of the results.
5. Vortex to mix thoroughly, then centrifuge briefly to the bottom of the tube.
6. Incubate at 60 - 65°C for 30 - 60 minutes.

B. pH indicators

Indicator	Suggested Concentration	pH Transition Range	Color Change (High pH to Low pH)
Phenol Red	0.5 mM to 5.0 mM	8.2 - 6.8	Red to Yellow
Cresol Red	0.05 mM to 1.5 mM	8.8 - 7.2	Red to Yellow
m-Cresol Purple	0.05 mM to 2.5 mM	9.0 - 7.4	Purple to Yellow
Thymol Blue	0.05 mM to 1.5 mM	9.6 - 8.0	Blue to Yellow

C. Cycling program*

Step	Cycles	Temp	Time
Isothermal Incubation	1	65°C	30 - 60 minutes
Heat Inactivation	1	80°C	5 minutes

* Lid temperature was set at 105°C.

Product Use Limitation
This product is for research purpose an *in vitro* use only