WizPure™ Bst DNA Polymerase (Large Fragment)

RUO For Research Use Only

REF W1321

DESCRIPTION

WizPure™ Bst DNA Polymerase (Large Fragment) is a DNA polymerase from *Bacillus stearothermophilus* that is modified so that it retains its 5'→ 3' DNA polymerase activity while lacking the intrinsic 5'→ 3' exonuclease domain. This polymerase also has strand displacement capabilities making it an ideal candidate for isothermal amplification.

KIT CONTENTS

Contents	W1321	W1321-5
Bst DNA Polymerase (LF), (8U/µl)	250 µl	1,250 µl
10X Bst Reaction Buffer	1 ml	5 x 1 ml
100mM MgSO4	1 ml	5 x 1 ml

STORAGE BUFFER

10mM Tris-HCl, 50mM KCl, 1.0mM dithiothreitol, 0.1mM EDTA, 0.1% Triton X-100, 50% glycerol, pH 7.5 (25°C)

10X BST REACTION BUFFER

200mM Tris-HCl, 100mM Ammonium Sulfate, 100mM KCl, 1.0% Triton X-100, pH 8.8 (25°C)

ACTIVITY

- 5'→3' exonuclease: No
- 3'→5' exonuclease: No
- Strand Displacement: Yes

APPLICATIONS

- DNA sequencing through high GC regions
- Rapid Sequencing from nanogram amounts of DNA template
- Loop-mediated isothermal amplification (LAMP)
- Whole genome amplification (WGA)
- Ramification amplification (RAM)

UNIT DEFINITION

1 unit is defined as the amount of polymerase required to convert 10 nmol of dNTPs into acid-insoluble material in 30 minutes at 65°C.

STORAGE CONDITIONS

Store all components at -20ºC in a non-frost-free freezer.

QUALITY CONTROL ANALYSIS:

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot of WizPure™ Bst DNA Polymerase (Large Fragment) is tested against predetermined specifications to ensure consistent product quality.

ORDERING INFORMATION

Product	Cat No.	Package
WizPure™ Bst DNA Polymerase (Large Fragment)	W1321	2,000 U
	W1321-5	10,000 U

PROTOCOL

1. LAMP Reaction Mixture Preparation

Prior to the experiment, it is prudent to carefully optimize experiment conditions and to include controls at every stage. See pre-protocol considerations for details.

For multiple reactions, scale up the volume of reaction components proportionally. All reagents can be prepared at room temperature.

1. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
2. The following table shows recommended component volumes:

Component	25 µl reaction	Final Conc.
Bst DNA Polymerase (LF)	1 - 2 µl	
10X Bst Reaction Buffer	2.5 µl	1X
dNTP mix (each 10mM)	3.5 µl	1.4 mM
100mM $MgSO_4$	2 - 2.5 µl	8 - 10 mM
FIP Primer (10 µM)	2 - 5 µl	0.8 - 2.0 µM
BIP primer (10 µM)	2 - 5 µl	0.8 - 2.0 µM
F3 primer (10 µM)	0.5 - 1 µl	0.2 - 0.4 µM
R3 primer (10 µM)	0.5 - 1 µl	0.2 - 0.4 µM
Loop F (10 µM)	1 - 2 µl	0.4 - 0.8 µM
Loop B (10 µM)	1 - 2 µl	0.4 - 0.8 µM
Green fluorescence dye (25X)*	1 µl	1X
Template DNA	1 - 5 µl	
Distilled water	up to 25 µl	

* For Real-time fluorescence assay only

3. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.

<u>For Conventional LAMP Assay</u>

1. Transfer tubes on ice into an isothermal instrument or water bath.
2. Incubation 62°C for 30-60 min.
   NOTE: Assay conditions may need to be optimized, depending on different primer and template combinations.
3. Run 5µ of the amplification product on a 1.5% agarose gel.

<u>For Conventional LAMP Assay</u>

1. Transfer tubes to a Real-time assay instrument (eg, Real-time PCR machine)
2. Real-time LAMP program setting and running as follows,
   - Incubation temperature: 62°C
   - Time: 30-60 min (Scan/1 min).
   - Detection channel: SYBR green (FAM)

Technical Support

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