EURx® MOLECULAR BIOLOGY PRODUCTS

Opti Bst DNA Polymerase (Large Fragment, exo⁻)

(Bacillus stearothermophilus)

Cat. No.	size
E1079-01	1600 units
E1079-02	8000 units

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 10 nmoles of total deoxyribonucleotide into acid-insoluble material in 30 minutes at 65°C.

Storage Conditions: Store at -20°C.

Large exonuclease 5'→3' free fragment of thermophilic Bst DNA Polymerase with strand displacement activity.

Description:

- Opti Bst DNA Polymerase is a moderately thermostable enzyme from *Bacillus stearothermophilus* genetically optimized for faster amplification and more flexible reaction conditions.
- Optimized for LAMP (Loop Mediated Isothermal Amplification)
- Enhanced dUTP incorporation compared to Bst DNA Polymerase.
- Ultrapure, recombinant protein.
- The enzyme replicates DNA optimally at 65°C.
- Catalyzes the polymerization of nucleotides into duplex DNA in the 5´→3´ direction in the presence of magnesium ions.
- Lacks the 5´→3´ exonuclease activity, while retaining the polymerase activity (1).
- Broad activity range; can replace mezophilic polymerases as well as synthesize DNA at high temperatures. Thus it is suitable for amplification of difficult DNA templates, including repetitive sequences, GC-rich regions and problematic secondary structures (2, 3).
- Can be heat inactivated at temperatures above 80°C.
- Active over wide range of reaction buffer conditions and magnesium ions concentrations.
- Used in isothermal DNA sequencing at elevated temperatures.
- Ideal for DNA synthesis reactions requiring strand displacement.

Storage Buffer:

10 mM Tris-HCl (pH 8.0 at 20°C), 50 mM KCl, 0.1 mM EDTA, 0.1% Tergitol™ TMN, 1 mM DTT and 50% (v/v) glycerol.

1X Opti Bst DNA Polymerase Reaction Buffer:

50 mM Tris-HCl (pH 8.9 at 20°C), 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 2 mM $MgSO_4$, 0.1% Tergitol™ TMN.

Quality Control:

All preparations are assayed for contaminating endonuclease, exonuclease, and single and double-stranded DNase activities.

References:

1. *Stenesh, J. and Roe, B.A. (1972) Biochim. Biophys. Acta. 272, 156-166.*
2. *Hugh, G. and Griffin, M. (1994) PCR Technology, p.p.228-229.*
3. *McClary, J. et al. (1991) J. DNA Sequencing and Mapping, p.p.173-180.*

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Protocol:

Typical LAMP (Loop-mediated isothermal amplification) reaction:

1. Reactions should be setup on ice. Pipet with sterile filter tips and make the reaction mix in an area separate from DNA preparation or analysis. No-template controls should be included in all amplification sets.
2. Prepare LAMP Primer Mix with all 4 or 6 (with Loop) primers. 10X Primer Mix should contain: 16 µM FIP, 16 µM BIP, 2 µM F3, 2 µM B3, 4 µM LoopF, 4 µM LoopB in TE or water.
3. Use a specific detection instrument for isothermal amplification or a real-time PCR cycler to run the assays. Real-time detection of the DNA amplification with a fluorescent dye is highly recommended.
4. Set the instrument to a constant incubation temperature between 60 to 65°C (depending on the primer annealing temperature). Measure the fluorescence intensity at an interval of 1 minute for up to 30 min.

	25 µl reaction	Final concentration
10X Opti Bst DNA-Polymerase Reaction Buffer	2.5 µl	1X (contains 2mM $MgSO_4$)
$MgSO_4$ (100 mM)	1.5 µl	6 mM (8 mM total)
dNTPs (10mM)	3.5 µl	1.4 mM each
10X LAMP Primer Mix	2.5 µl	1X
Opti Bst DNA Polymerase (8 U/µl)	1 µl	0.32 U/µl
DNA sample	variable	100 copies or more
PCR-grade water	up to 25 µl	

Additional information:

1. If reaction optimization is needed:
   - Change Mg^{2+} concentration in a range of 4—10 mM.
   - Choose a different enzyme concentration (0.040—0.4 U/µl).
   - Set other reaction temperature (50 to 68°C).
   - Extend the reaction time. (Prelonged incubation may cause amplification of unspecifically annealed primers or primer dimer formations).
1. For difficult DNA templates pre-warm the reaction mix **witout enzyme** at 92°C for 1 min. Cool down to room temperature, then add the Opti Bst DNA Polymerase, mix well, centrifuge and place tubes into a pre-heated thermocycler or heat block.
2. If amplification in no-template controls occurs the following points should be reviewed.
   - Replace reagent stocks and pre-mixes with new components.
   - Use separate preparation area and equipment if post-reaction processing is necessary.
   - Avoid opening reaction tubes after amplification.
   - Use dNTPs with 50% inclusion of dUTP mixed with dTTP and 0.2 U of Thermolabile Uracil-N-glycosylase (E1251) per reaction.

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