TargetEx Bst Polymerase (exo-) 5 mg/mL

Catalogue Number: TGX-004-BS/-BM/-BL

INTRODUCTION

TargetEx Bst Polymerase (exo-) is the large fragment of a thermostable enzyme derived from the bacterium *Geobacillus stearothermophilus* (formerly: *Bacillus stearothermophilus*). This enzyme is ideal for highly efficient isothermal amplifications such as Loop-mediated Isothermal Amplification (LAMP) or Recombinase Polymerase Amplification (RPA).

PREPARATION OF A LAMP REACTION MIX

Prepare a 10-50-fold* enzyme dilution from the 5 mg/mL polymerase stock with 10 mM Tris, 100 mM KCl, 0.1 mM EDTA, pH=7.5 buffer. Use 1 µL of this enzyme dilution for a 20 µL LAMP reaction.

*Optimization may be required

Store the product at -20 °C. Avoid freeze-thaw cycles.

TYPICAL REACTION ASSEMBLY

To use the enzyme in a LAMP reaction with a qPCR instrument, prepare a 10X Primer Mix using the following components with the suggested final concentrations: FIP (16 µM), BIP (16 µM), F3 (2 µM), B3 (2 µM), LoopF (4 µM), and LoopB (4 µM). Mix all the listed components (see below), then remove all visible bubbles by centrifuging the plate or tubes. Create a heat profile on your thermal cycler according to the thermal cycler program setup below (in LAMP applications using qPCR machines, fluorescence is often measured cyclically with 15 s incubation at 65 °C followed by readout, repeated up to 60-80 times). Select SYBR channel for fluorescence readout when using SYTO9 stain. For reliable results, we recommend including technical replicates and obtaining a melt curve.

COMPONENT LIST

Materials	Volume to 20 µL (single measurement)	Recommended final concentration
Nuclease-Free Water (NFW)	To 20 µL	N/A
10X Reaction buffer*	2 µL	1X
10X Primer Mix	2 µL	1X
dNTP mix (14 mM each)	2 µL	1.4 mM
DNA Template	Variable	Variable**
Fluorescent dye (e.g.: SYTO9)	1 µL	0.4 µM
Diluted TargetEx Bst Polymerase (exo-)	1 µL	0.1-0.5 µg/reaction

*E.g.:30-100 mM $MgSO_4$, 200 mM Tris, 100 mM $(NH_4)_2SO_4$, 1% Tween-20, 500 mM KCl, pH=8.8
**We recommend using more than 1 pg DNA/reaction

THERMAL CYCLER PROGRAM SETUP

No.	Step	Temperature (°C)	Duration	Number of Cycles
1	Amplification	65	15 sec	60-80
2	Inactivation	95	2 min	1
3	Melt Curve	60-95	Variable	Variable

If you need further information or would like to try our products, please do not hesitate to contact us at **info@targetex.com**.

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LAMP TROUBLESHOOTING GUIDE:

No amplification signal:

- Verify the concentration of your fluorescent dye. For SYTO dyes, a final concentration of 0.2-1.0 µM is recommended.
- The primer set may not efficiently recognize the target sequence. Consider redesigning primers and verifying the target sequence conservation.
- Confirm that the final dNTP concentration is within 0.5-2.0 mM. Prepare the dNTP mix fresh whenever possible and avoid repeated freeze - thaw cycles.
- An insufficient or degraded template may result in no amplification. Verify template quality and concentration.
- Check that the reaction temperature and incubation time are appropriate for the enzyme used.
- Avoid repeated freeze-thaw cycles of enzyme and reaction components.

Amplification detected in the NTC (No Template Control):

- Cross-contamination is the most common cause of false positives. Use filter tips, dedicated work areas, and separate pre- and post-amplification workflows.
- Excessively long incubation times may promote non-specific amplification; reduce reaction time if late NTC signals are observed.
- Never reuse reaction plates or tubes. Do not open reaction vessels after amplification whenever possible.
- Optimize the Mg^{2+} concentration within the range of 3-10 mM/reaction.
- Analyze amplification products in a separate room from reaction setup if possible.
- Always work from aliquots to reduce the risk of contaminating stock solutions. Avoid repeated use of large-volume stock reagents whenever possible.
- Review the melt curve analysis. Unexpected or multiple peaks may indicate non-specific amplification.
- Consider redesigning primers if non-specific amplification persists.
- Optimize the final dNTP concentration within the range of 0.5-2.0 mM.