Bst X DNA Polymerase

Key benefits

- Improved amplification speed and sensitivity compared to standard Bst DNA Polymerase
- Provides rapid LAMP detection of DNA targets over a wide range of enzyme concentrations
- Exhibits strong strand displacement activity with no 5'→3' and no 3'→5' exonuclease activity
- Enables fast amplification of targets from both simple and complex genomic DNA templates at high and low copy numbers
- Displays high salt tolerance and functions in the presence of detergent
- Heat stable with an optimal reaction temperature range of 60–70°C; inactivated at temperatures greater than 75°C

QIAGEN Bst X is a recombinant, thermostable DNA polymerase derived from *Geobacillus*. Bst X is homologous to Bst, the DNA polymerase derived from *Bacillus stearothermophilus* and, like Bst, lacks 5'→3' and 3'→5' exonuclease activity and has a strong displacement activity. Compared to Bst DNA polymerase, Bst X displays improved amplification speed, salt tolerance and reaction sensitivity.

Isothermal amplification sensitivity

Loop-mediated isothermal amplification (LAMP) is a common method for accurate and cost-effective detection of a variety of pathogens, particularly in point-of-care environments (1). Figure 1 demonstrates that Bst X amplifies targets rapidly from both simple and complex genomic DNA templates at high and low target copy numbers. The absence of amplicon accumulation in non-template control (NTC) reactions reflects both the specificity of the reaction and the absence of exogenous contaminating DNA in purified Bst X.

Rapid DNA detection

Bst X accelerates DNA detection response time when compared to other enzymes commonly used in LAMP assays. This is an important feature for point-of-care detection, where time-to-result is crucial. Figure 2 presents a comparison of Bst X with two commercially available Bst DNA polymerases in LAMP assays. In experiments using several different templates and targets, Bst X consistently gave faster response times across a range of target input concentrations when compared to competitive enzymes.

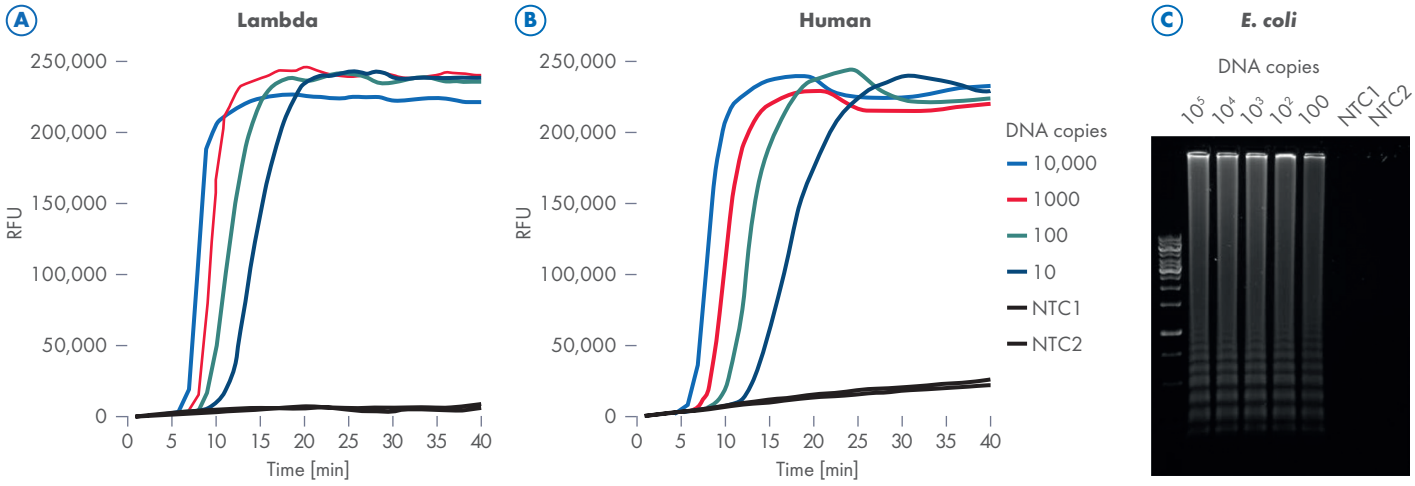

Figure 1

LAMP reactions were performed at 65°C using 40 U of Bst X and primer sets targeting lambda phage, human (CTFR) or *E. coli* (malB) over a range of DNA template copies from 10,000 to as little as 10 in a 25 µL volume. **A-B** EvaGreen® dye was added to reactions and fluorescence was monitored using a real-time PCR instrument (Applied Biosystems®). **C** Reactions were stopped after 30 minutes and products were analyzed by agarose gel electrophoresis and ethidium bromide staining.

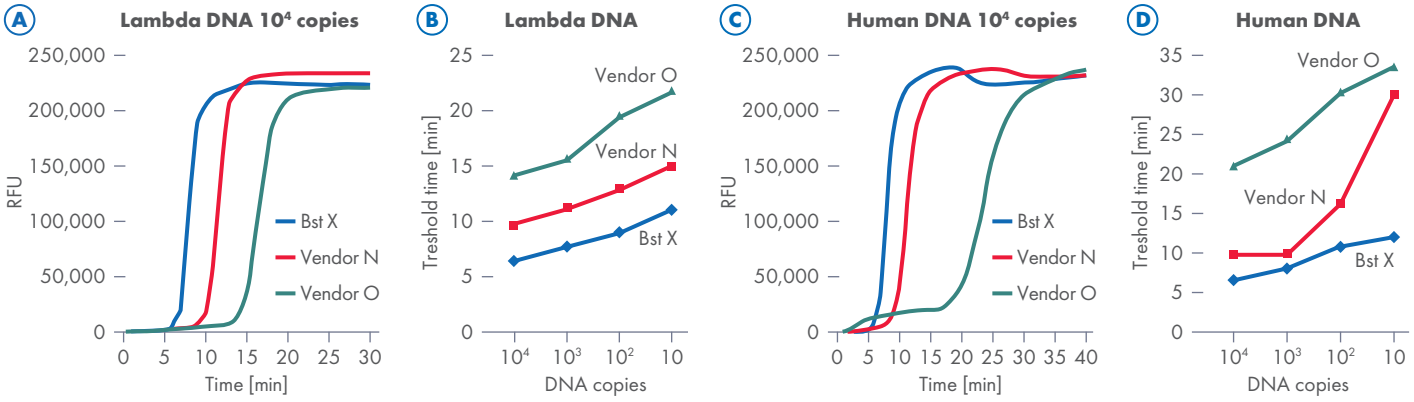

Figure 2

LAMP assays (25 µL) targeting lambda phage or human DNA (CTFR) were performed in-house using 40 U of Bst X or a matching quantity of competitive enzyme. In panels **A** and **C**, fluorescence amplification curves are shown starting with a fixed quantity of 10^4 copies. In panels **B** and **D**, the calculated fluorescence threshold times were determined using real-time PCR software (Applied Biosystems) from reactions containing a range of template concentrations.

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Flexible reaction setup

While the fastest LAMP detection times are achieved using a relatively high concentration of polymerase, this can also cause accelerated NTC detection, potentially leading to false positive results. The time gap between target and NTC detection time can vary significantly depending on factors such as primer design, temperature and the concentrations of enzyme and magnesium.

Figure 3 presents data that Bst X provides rapid LAMP detection of DNA targets over a wide range of enzyme concentrations, providing flexibility in the time delay between template-directed nucleic acid detection and the amplification of spurious molecules.

Robust performance

Bst X displays a higher degree of salt tolerance as c ompared to Bst with little change in detection time at salt concentrations up to approximately 125 mM (Figure 4).

Additionally, Bst X was shown to be resistant to Triton-X, NP-40, Tween-20 and Tween-80 detergents at final concentrations up to 5% when tested in LAMP reactions (data not presented).

Reaction conditions

Bst X has an optimal reaction temperature range of 60–70°C. Rapid inactivation occurs at temperatures greater than 75°C. If desired, Bst X activity can be effectively eliminated by heat treatment at 80°C for 10 minutes.

Bst X is provided with proprietary 10x Xcelerator Reaction Buffer containing 2 mM $MgSO_4$ when diluted to 1x. An additional tube of 100 mM $MgSO_4$ is provided to allow users to optimize conditions in their applications of interest.

Quality and service you can count on

QIAGEN manufactures pure, superior enzymes and reagents for molecular biology and other applications. QIAGEN strives to resolve its customers' challenges by providing the highest quality materials, an unbreakable supply chain and paradigm-shifting service. With a manufacturing record unmatched in commercial enzyme production, QIAGEN designs analytical grade quality into all its products to meet the most rigorous specifications.

Our elite-grade Bst X DNA Polymerase reflects our commitment to identifying, developing and delivering the very best in enzyme technologies. If your company requires products and a service partner that stand above the crowd, we'd love to hear from you.

Some applications of Bst X may be covered by issued patents. Purchase of this product does not include a license to any patented applications, and it is the users' sole responsibility to determine whether they may be required to obtain a license for their intended application.

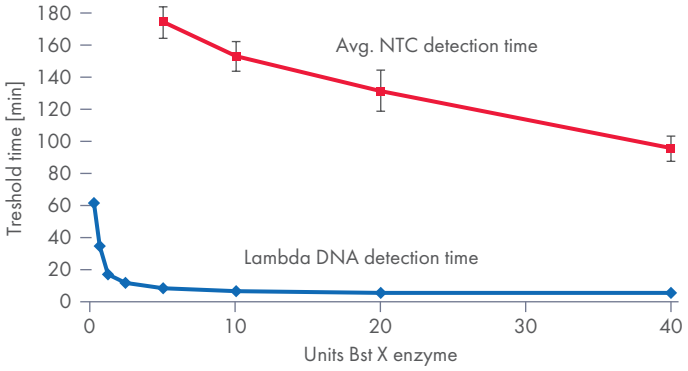

Figure 3

LAMP reactions (25 µL) targeting lambda DNA were set up using a range of Bst X concentrations with either 10^4 copies of lambda DNA or with no template DNA, as indicated. For NTC reactions, the average threshold time of triplicate reactions was determined, and the standard deviation is indicated by error bars.

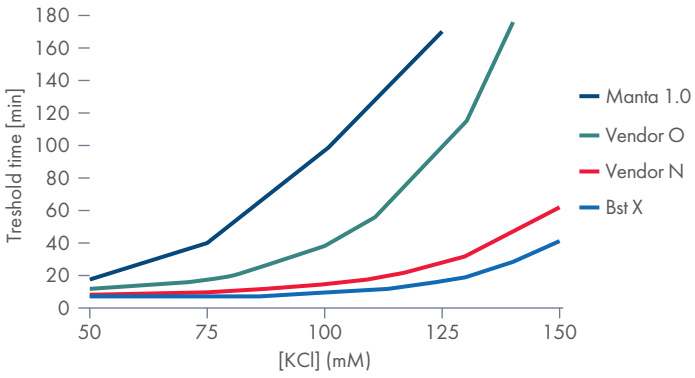

Figure 4

LAMP reactions (25 µL) targeting lambda DNA (10^5 copies) were performed over a range of KCl concentrations using 40 U of Bst X, 40 U of QIAGEN Manta 1.0 DNA polymerase, or a matching quantity of competitive enzyme. Fluorescence threshold times were calculated using real-time PCR software (Applied Biosystems).

Ordering Information

Product	Contents	Cat. no.
Bst X DNA Polymerase	Bst X DNA Polymerase (1.0 mL at 40,000 U/mL) and 10x Xcelerator Reaction Buffer (1 x 7.0 mL) and 100 mM Magnesium Sulfate Solution (1 x 7.0 mL)	P7390L

References

1. Notomi T, et al. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research 2000*; **28**: e63

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