SynBst Polymerase

Polymerase for isothermal amplification

CAUTION! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from synthbioenzymes.com.

The protocols and storage conditions detailed below ensure that the SynBst Polymerase can function at its highest potential. These guidelines cover standard amplification protocols for all LAMP reactions and unique specifications tested to provide optimal conditions for SynBst Polymerase.

1. Product description

SynBst is the engineered large fragment of *Bacillus stearothermophilus* DNA polymerase. It's a thermotolerant strand-displacement polymerase lacking 5'-3'- and 3'-5'-exonuclease activity. Strand-displacing polymerases are a crucial component of isothermal amplification reactions, where the lack of thermal cycling reduces equipment needs and improves the time to answer.

Its fast amplification and very robust strand displacement activity makes SynBst a perfect candidate for special PCR applications like isothermal amplification (LAMP), whole genome amplification (WGA), multiple displacement amplification (MDA). SynBst also works efficiently with multiple factors such as high GC content, secondary structures in template, and low template concentration.

2. Contents

SynBst package information

B001M	1600 U (8 U/µl) = 200 x 25 µl reactions Material provided: SynBst Polymerase 1600 U (200 µl), 10x SynBst Polymerase Buffer (1.5 ml), and 100 mM $MgSO_4$ solution (1.5 ml)
B001L	8000 U (8 U/µl) = 1000 x 25 µl reactions Material provided: SynBst Polymerase 2000 U (1 ml), 10x SynBst Polymerase Buffer (2 x 1.5 ml), and 100 mM $MgSO_4$ solution (2 x1.5 ml)

3. Storage conditions for SynBst Polymerase and Buffer

Although our experiments confirm that the SynBst Polymerase is viable after incubation at room temperature or 4°C for 2 weeks, we recommend storing all components at -20°C to ensure its quality.

4. Guidelines for using SynBst Polymerase

All reaction preparations should be done over ice with sterile lab equipment. All components of the reaction mix should be mixed and centrifuged gently.

4.1 Standard LAMP reaction protocol

Primers We recommend using online programs to design primers suitable for the LAMP reaction. These programs analyze the desired template and determine the optimal length, GC content and melting temperature. We recommend PrimerExplorer for Loop-mediated isothermal amplification (LAMP) and Primer3web for other isothermal amplification-based reactions like whole genome amplification. Ordering oligonucleotides in different purification can increase the efficiency of the LAMP reaction, but experiments show that the standard desalted primers suffice.

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It is important to make a primer mastermix for the reaction, in which the concentration of each primer should be the following (for standard LAMP reaction):

Component	Final concentration in the 10x Mastermix
FIP Primer (Forward inner primer)	16 µM
BIP Primer (Backward inner primer)	16 µM
F3 Primer (Forward outer primer)	2 µM
B3 Primer (Backward outer primer)	2 µM
LoopF Primer	4 µM
LoopB Primer	4 µM

The recommended reaction protocol for the SynBst Polymerase is the following.

Component	25 µl reaction
SynBst Buffer (10x)	2.5 µl
dNTP(10mM)	3.5 µl
SynBst Polymerase	1 µl
Primer Mix (10x)	2.5 µl
DNA/RNA sample (>10 copies)	variable
$MgSO_4$ (100mM)	1 µl
H_2O	to 25 µl

Incubate the reaction mixture at 65°C for 30-60 minutes. We recommend fluorescent detection every 30 seconds.

4.2 Additives

Although several additive substances were tested which can improve the amplification time of the LAMP reaction, - the most commonly used being the Guanidine hydrochloride -, the SynBst Polymerase have been engineered to be more effective than the general Bst polymerases with guanidine hydrochloride added to their reaction mixture. As the SynBst polymerase is more optimized than other Bst Polymerases we **DO NOT** recommend using Guanidine hydrochloride as an additive substance as it will not improve the amplification time.

The concentration of magnesium is important for the LAMP reaction to take place, as it is the cofactor of the polymerase enzyme. Therefore, the concentration of Mg^{2+} significantly affects the activity of the enzyme and the PCR reaction. If optimization is desired raise the final Mg^{2+} concentration within the 4–10 mM range. Note that excess Mg^{2+} can also stabilize spurious annealing of primers to incorrect template sites and decrease specificity.

5. Troubleshooting

Visit our online FAQ for tips and tricks and troubleshooting information:

https://synthbioenzymes.com/product/synbst-polymerase/