Check the product label for actual catalog number, lot and expiry date.

ALLin™ Hot Start Bst Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
IDE030E	1600 u	1600 u – ALLin™ Hot Start Bst Polymerase, 8 u/µl 1 x 0.5 ml – 10X ALLin™ Bst Buffer 1 x 1 ml – 5X Bst Enhancer	Glycerol-free formulation of the recombinant Hot Start Bst DNA Polymerase (large fragment) in storage buffer. Excellent for both common and lyo-ready workflows. ALLin™ Bst Buffer contains optimal concentration of dNTPs and magnesium sulphate as well as stabilizers. The proprietary Bst Enhancer accelerates the reaction.
IDE0301	8000 u	8000 u – ALLin™ Hot Start Bst Polymerase, 8 u/µl 2 x 1.25 ml – 10X ALLin™ Bst Buffer 3 x 1.7 ml – 5X Bst Enhancer	
Storage		*In the dark at -20°C. As the enzyme is glycerol-free, to avoid multiple freezing-thawing, aliquot the enzyme into 5-10 sterile tubes.*	

APPLICATIONS

- Isothermal DNA amplification at elevated temperature
- Real-time detection of DNA amplification
- MDA – multiple displacement amplification
- LAMP - loop-mediated isothermal amplification
- WGA - whole genome amplification
- RAM - ramification & RPA - recombinase polymerase ampl.

BENEFITS

- Glycerol-free formulation of robust Hot Start Bst DNA Polymerase, supplied with all-included buffer with dNTPs and proprietary reaction enhancer
- The enzyme is blocked at ambient temperature due to molecular inhibition based hot-start technology, what is excellent for ambient reaction setup
- Active at high temperatures in a range of 55-70°C
- Fast DNA amplification at constant temperature
- Ideal for complex templates and crude samples
- Ensures <3 molecules LOD (limit of detection)

PRODUCT DETAILS

The ALLin™ Hot Start Bst Polymerase is a recombinant protein, representing a large fragment of the *B. stearothermophilus* DNA Polymerase expressed in *E. coli* cells. The activity of the enzyme is blocked at ambient temperature due to molecular inhibition based hot-start technology, and the polymerase is activated only at 45-50°C what reduces non-specific amplification, primer dimer formation and is therefore excellent for ambient reaction setup. This robust polymerase with a strong strand displacement activity and high temperature tolerance ensures high amplification yield at constant temperature when working with impure or low-copy number targets as well as with complex templates. The Allin™ Bst Buffer includes optimal concentrations of magnesium and dNTPs, what minimizes pipetting steps. This ALLin™ Hot Start Bst Polymerase-buffer system together with a supplied enhancer, detects <3 DNA targets in a short time without the use of a thermocycler. This glycerol-free Hot Start Bst Polymerase is also an excellent tool for development of dry format lyophilized kits for pathogen detection using isothermal amplification techniques.

PERFORMANCE

For more convenience, we recommend our kit formulations:
ALLin™ HS Isothermal DNA Amplification Kit and ALLin™ HS Iso-Colorimetric DNA Amplification Kit both including 2X master mix formulations.
Technical characteristics of Hot Start Bst Polymerase (large fragment):

- The polymerase is blocked at ambient temperature and activated at temperatures above 45°C.
- Strong 5' - 3' strand displacement activity
- Strong 5' - 3' polymerase activity
- No 5' - 3' exonuclease activity
- No 3' - 5' exonuclease (proofreading) activity
- Only minor reverse transcriptase activity
- Optimal amplification temperature is 65°C
- Working temperature range is 55-70°C
- Optimal reaction time is 20 minutes (depends on the buffer)
- If needed, the reaction can be extended to 30 - 60 minutes
- The enzyme is inactivated in 10 minutes at 80°C

The use of this product in certain applications may be covered by patents of third parties. The user has to analyse all applicable Limited Use Label Licenses and may need licensing from third parties.

ISOTHERMAL AMPLIFICATION PROTOCOL EXAMPLE

- Take typical measures to prevent contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive controls in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Ambient temperature assembly can be performed with the HS Bst, as the enzyme becomes activated only above 45°C.
- Perform the reaction at 65°C. If needed, optimize the reaction temperature between 55-70°C for each template/primers system. Complex templates may require higher temperature.
- Suggested reaction time is 20-30 minutes. For some low copy number targets 30-60 minutes might be required.
- Design primers with predicted melting temperature of about 60°C.
- Prepare 10X primer mix in water or TE Buffer, for example, for LAMP: 16 µM FIP, 16 µM BIP, 2 µM F3, 2 µM B3, 8 µM LoopF, 8 µM LoopB.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 25 µl reaction:

	µl

✓ Mix gently, avoid bubbles.

✓ Place into the thermostat or qPCR instrument to incubate:

	55-70°C, time between 20 - 60 min
Optional: Inactivation	80°C - 10 min

High yield amplification product may increase the viscosity of the solution, mix well and dilute if needed for downstream applications.

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