PCRBIOSYSTEMS
simplifying research

www.pcrbio.com

IsoFast™ Bst Polymerase

Product description

IsoFast™ Bst Polymerase is a recombinant protein expressed in E. coli and represents the large fragment of *Geobacillus stearothermophilus* (formerly known as *Bacillus stearothermophilus*) DNA Polymerase. This portion of the protein maintains the protein 5' to 3' polymerase activity but lacks the 5' to 3' exonuclease activity.[1]

IsoFast™ Bst Polymerase displays strong strand displacement activity and is suitable for nucleic acid amplification methods such as whole genome amplification, multiple displacement amplification and isothermal amplification. We recommend a reaction temperature of 65ºC, however the enzyme works well over a broad temperature range, from 55ºC to 70ºC. It is heat inactivated at 80ºC.

Designed for fast amplification speed, IsoFast™ Bst Polymerase gives rapid and consistent results across different target sequences and sample types. The enzyme is provided with a 2-part buffer system to ensure high yield and performance even under difficult conditions.

Real-time detection with any qPCR thermocycler can be achieved by adding 20x Fluorescent Dye to the reaction. This is supplied separately, catalogue number PB80.30-2 and PB80.30-10.

1. Mead DA, McClary JA, Luckey JA, Kostichka AJ, Witney FR, Smith LM. Bst DNA polymerase permits rapid sequence analysis from nanogram amounts of template. Biotechniques. 1991 Jul;11(1):76-8, 80, 82-87.

Component	1600 units	8000 units
IsoFast Bst Polymerase (8U/µL)	1 x 200µL	1 x 1mL
10x IsoFast Buffer A	1 x 500µL	2 x 1.25mL
5x IsoFast Buffer B	1 x 1mL	3 x 1.7mL

Shipping and storage

On arrival the kit should be stored between -30˚C and -15˚C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months.

We recommend aliquoting the enzyme at the first use to avoid more than 10 freeze/thaw cycles. All the other components of the kit can go through 30 freeze/thaw cycles with no loss of activity.

Limitations of product use

The product may be used for in vitro research purposes only.

Technical support

Help and support is available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions. For technical support and troubleshooting you can submit a technical enquiry online, or alternatively email technical@pcrbio.com with the following information:

- Amplicon size
- Reaction setup
- Reaction conditions
- Screen grabs or images of amplification results

Important considerations

10x IsoFast Buffer A: The 10x buffer contains 30mM $MgSO_4$, 16mM dNTPs, enhancers and stabilizers. The buffer composition has been optimised to maximise the rate of amplification.

5x IsoFast Buffer B: The 5x buffer contains enhancers designed to further increase the reaction speed.

Primers: Primers should have a predicted melting temperature of around 60˚C, using default Primer Explorer v5 settings (http://primerexplorer.jp/lampv5e/index.html). The final primer concentration in the reaction should be 0.2µM for F3 and B3 primers, 1.6µM for FIP and BIP primers and between 0.4 and 0.8µM for LoopF and LoopB primers.

Example usage: Strand displacement

Reaction temperature	Reaction time	Deactivation temperature	Deactivation time
Recommended: 65˚C Optimal range: 55-70˚C	30-60 minutes	80˚C	10 minutes

Example usage: Loop-mediated isothermal amplification (LAMP)

1. Allow each component to reach room temperature, then briefly vortex.
2. Prepare a master mix based on the following table. Reactions should be set up on ice:

Reagent	25µL reaction	Final concentration	Notes
10x IsoFast Buffer A	2.50µL	1x	
5x IsoFast Buffer B	5.00µL	1x	
20x Fluorescent Dye (optional)	1.25µL	1x	Available separately, catalogue number PB80.30-02 and PB80.30-10
IsoFast Bst Polymerase (8U/µL)	1.00µL	8U	
10x Primer set	2.50µL	1x	A primer set can be prepared with all 4 or 6 (if you include Loop) primers. A 10x primer set should contain: 16µM FIP, 16µM BIP, 2µM F3, 2µM B3, 4-8µM LoopF, 4-8µM LoopB in TE Buffer or water.
Template DNA	Variable		
PCR grade dH_2O	Up to 25µL final volume		

3. Incubate at 65˚C for 30 minutes. Time can be extended and temperature can be modified (between 55˚C and 70˚C) as necessary for low copy targets, challenging templates, or whenever amplification times have been reported to be slow.

   If a qPCR instrument is used for signal detection, follow the reaction using the FAM channel, acquiring data every 10-15 seconds. If final products are to be analysed after the reaction is complete, the enzyme can be inactivated by heating at 80˚C for 10 minutes.

witec ag
experts in life science products
Industriestrasse 12 | 6210 Sursee
T 041 250 53 57 | mail@witec.ch