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IsoFast™ Hot Start Bst Polymerase with Dye

Product description

The product contains IsoFast™ Hot Start Bst Polymerase 8 U/µL, reaction buffers, and 20x Fluorescent Dye for DNA detection.

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

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

PCRBIO's innovative AptaLock™ technology uses proprietary aptamer-like molecules that reversibly inhibit Bst Polymerase at room temperature, reducing non-specific amplification prior to amplification at >45-50 °C. This unique hot start effect reduces primer dimer formation and nonspecific amplification and helps to maximise the sensitivity and specificity of the reaction.

Designed for fast amplification speed, IsoFast™ Hot Start Bst Polymerase gives rapid and consistent results across different target sequences and sample types. The kit includes an advanced buffer system to ensure high yield and performance even under difficult conditions.

Real-time detection with any qPCR thermocycler can be achieved by adding the supplied 20x Fluorescent Dye to the reaction.

Component	1600 Units	8000 Units
IsoFast Hot Start Bst Polymerase 8 U/µL	1 x 200 µL	1 x 1 mL
10x IsoFast Buffer A	1 x 500 µL	2 x 1.25 mL
5x IsoFast Buffer B	1 x 1 mL	3 x 1.7 mL
20x Fluorescent Dye	2 x 125 µL	2 x 625 µL

Shipping and storage

On arrival the kit should be stored between -30 °C and -15 °C. Avoid prolonged exposure to light and keep components on ice when in use. If stored correctly the kit will retain full activity for 12 months. We recommend aliquoting theenzyme upon first use to avoid excess freeze/thaws.

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.

References

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.

Important considerations

10x IsoFast Buffer A: The 10x buffer contains 30 mM $MgSO_4$, 16 mM 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.

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 components to reach room temperature, then briefly vortex.
2. Prepare a master mix based on the following table. Reactions may be set up on ice:

Reagent	25 µL reaction	Final concentration	Notes
10x IsoFast Buffer A	2.50 µL	1x	
5x IsoFast Buffer B	5.00 µL	1x	
20x Fluorescent Dye (optional)	1.25 µL	1x	
IsoFast Hot Start Bst Polymerase (8 U/µL)	1.00 µL	8 U	
10x Primer set	2.50 µL	1x	We recommend a predicted melting temperature of around 60 ˚C using default Primer Explorer v5 settings. A primer set can be prepared with all 4 or 6 (if you include Loop) primers. A 10x primer set should contain: 16 µM FIP, 16 µM BIP, 2 µM F3, 2 µM B3, 4-8 µM LoopF, 4-8 µM LoopB in TE Buffer or water.
Template DNA	Variable		
PCR grade dH_2O	Up to 25 µL 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.

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