



PCRBIOSYSTEMS

simplifying research

## IsoFast™ Hot Start Bst Mix

[www.pcrbio.com](http://www.pcrbio.com)

### Product description

IsoFast™ Hot Start Bst Mix is a convenient, ready-to-use 2x mix containing the versatile IsoFast™ Hot Start Bst Polymerase enzyme combined with dNTPs and enhancers in reaction-ready format.

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.<sup>1</sup>

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 Mix works well over a broad temperature range, from 55 °C to 70 °C. It can be heat inactivated at 80 °C.

PCR BIO's innovative AptaLock™ technology uses proprietary aptamer-like molecules that reversibly inhibit Bst Polymerase at room temperature, reducing non-specific amplification prior to activation 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 speeds, IsoFast™ Hot Start Bst Mix gives rapid and consistent results across different target sequences and sample types. The mix 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	100 Reactions	500 Reactions
2x IsoFast™ Hot Start Bst Mix	1 x 1.25 mL	5 x 1.25 mL
20x Fluorescent Dye	1 x 125 µL	1 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 the fluorescent dye 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](mailto: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, Whitney 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

**2x IsoFast Hot Start Bst Mix:** The 2x mix contains 6 mM MgSO<sub>4</sub>, 3.2 mM dNTPs, enhancers and stabilizers and IsoFast™ Hot Start Bst Polymerase. The mix composition has been optimised to maximise the rate of amplification.

## 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	25µL reaction	Final concentration	Notes
2x IsoFast Bst Mix	12.50 µL	1x	
20x Fluorescent Dye (optional)	1.25 µL	1x	
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 <sub>2</sub> O	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.

## Notice to purchasers

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