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IsoFast® Hot Start Bst Polymerase Colour

Product description

IsoFast® Hot Start Bst Polymerase Colour comprises IsoFast® Bst Polymerase combined with AptaLock™ hot start technology and supplied with a proprietary buffer for colourimetric detection of DNA targets.

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 5' to 3' polymerase activity but lacks 5' to 3' exonuclease activity.¹ Hot start reversibly inhibits Bst Polymerase at room temperature, reducing non-specific amplification below 45-50 °C.

The colour buffer system allows pH-based colourimetric detection of amplicons. Colour will switch from orange (negative, no template control samples) to yellow (positive sample) when amplification takes place.

¹Mead 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.

Quality control

PCR Biosystems operates under an ISO 13485 certified Quality Management System. Our products are extensively tested and undergo a comprehensive, multi-step quality control process according to ISO 13485 standards, to ensure optimum performance, consistency and traceability.

Component	1600 Units	8000 Units
IsoFast® Hot Start Bst Polymerase 8 U/μL	1 x 200 μL	1 x 1 mL
10x IsoFast® Colour Buffer A	1 x 500 μL	2 x 1.25 mL
5x IsoFast® Buffer B	1 x 1 mL	3 x 1.7 mL

Shipping and storage

On arrival the kit should be stored between -30 °C and -20 °C. If stored correctly, the kit will retain full activity until the indicated expiry date. Keep components on ice when in use. Avoid exposure of the stock solution to frequent temperature changes and limit handling at room temperature to the necessary minimum.

Technical support

Scan or click the QR code for answers to frequently asked technical questions. For further technical support, please email technical@pcrbio.com with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile



FAQS

Product Use: Unless we agree otherwise in writing, the Goods we supply are provided:

1. For research purposes only and you should not use or rely on the Goods for diagnostic purposes. If you wish to use the Goods in a regulatory approved medical device, please contact us so that we may consider this and discuss it further with you.
2. Subject to our standard terms and conditions found at <https://pcrbio.com/terms-conditions/>.

Important considerations

10x IsoFast Colour Buffer A: The 10x buffer contains 30 mM MgSO₄, 16 mM dNTPs, enhancers and stabilizers. The buffer composition has been optimised to maximise the rate of amplification. The buffer also contains a pH-sensitive dye, enabling the detection of amplification without any instruments. For examples of the expected colour shift, please refer to our product page online.

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

Reaction Temperature: We recommend a reaction temperature of 65 °C. However, IsoFast® Hot Start Bst Polymerase Colour works well over a broad temperature range, from 55 °C to 70 °C. It can be heat inactivated at 80 °C.

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	25 µL reaction	Final concentration	Notes
10x IsoFast Buffer A	2.5 µL	1x	
5x IsoFast Buffer B	5 µL	1x	
IsoFast® Hot Start Bst Polymerase (8 U/µL)	1 µL	8 U	
10x Primer set	2.5 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 ₂ 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.

Colour output can be assessed visually or using a plate-reader spectrophotometer. 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.

Real time detection via addition of a fluorescent dye is not recommended with this mix. For this type of detection catalogue numbers PB80.40, PB80.41, and PB80.42 are preferable.

Notice to purchasers

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