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IsoFast™ Hot Start Bst Colour Mix

Product description

IsoFast™ Hot Start Bst Colour Mix comprises IsoFast™ Bst Polymerase combined with AptaLock™ hot start technology in a proprietary mix 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]

IsoFast™ Hot Start Bst Polymerase displays strong strand displacement activity and is suitable for a wide range of isothermal amplification workflows. We recommend a reaction temperature of 65 ˚C. However, the enzyme 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 Colour Mix 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.

Component	100 Reactions	500 Reactions
2x IsoFast Hot Start Bst Colour Mix	1 x 1.25 mL	5 x 1.25 mL

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).

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.

Shipping and storage

On arrival the kit should be stored between -30 ˚C and -15 ˚C. Keep components on ice when in use. If stored correctly the kit will retain full activity for 12 months. We recommend aliquoting the enzyme 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

Important considerations

2x IsoFast Hot Start Bst Colour Mix: The 2x mix contains 6 mM $MgSO_4$, 3.2 mM dNTPs, enhancers and stabilizers, a pH-sensitive dye, and IsoFast™ Hot Start Bst Polymerase. The mix composition has been optimised to maximise the rate of amplification and ensure an orange to yellow colour shift when DNA targets are amplified with this mix. For examples of the expected colour shift, please refer to our product page online.

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
2x IsoFast Hot Start Bst Colour Mix	12.50 µL	1x	
20x Fluorescent Dye (optional)	1.25 µL	1x	Available separately, catalogue number PB80.30-02 and PB80.30-10
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.

   After run, positive samples will appear yellow, while negative samples will appear dark pink-orange.

   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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