PCRBIOSYSTEMS
simplifying research

www.pcrbio.com

IsoFast™ Bst 1-Step Mix

Product description:

IsoFast™ Bst 1-Step Mix is a dual enzyme system designed for rapid and sensitive reverse transcription of target RNA and subsequent isothermal amplification in a single tube.

The kit utilises IsoFast™ Bst Polymerase (in a 2x mix format) for its strong strand displacement activity, enabling DNA synthesis at a constant temperature without the need for thermal cycling. Representing the large fragment of *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) DNA Polymerase, this portion of the protein catalyses the 5'-3' synthesis of DNA but does not contain the 5'-3' exonuclease domain.[1]

Reverse transcription of target RNA is carried out by the thermostable and extremely active RTase Go, which is blended with RNase inhibitor to prevent degradation of RNA by contaminating RNase.

IsoFast Bst 1-Step Mix includes an advanced buffer system to ensure high yield and performance even under difficult conditions, such as the presence of inhibitors. The kit requires only the addition of primers, template and water, and includes separate fluorescent dye to allow real-time detection with any qPCR instrument.

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	100 reactions	500 reactions
2x IsoFast Bst Mix	1 x 1.25mL	4 x 1.56mL
20x Fluorescent Dye	1 x 125µL	1 x 625µL
RTase Go	1 x 200µL	1 x 1.00mL

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 fluorescent dye 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

2x IsoFast Bst Mix: The 2x mix contains 6mM $MgSO_4$, 3.2mM dNTPs, enhancers and stabilizers and IsoFast Bst Polymerase. The mix composition has been optimised to maximise the rate of amplification.

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 with IsoFast Bst Mix

Reaction temperature	Reaction time	Deactivation temperature	Deactivation time
Recommended: 65˚C	30-60 minutes	80˚C	10 minutes

Example usage: RT loop-mediated isothermal amplification (RT-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 Bst Mix	12.50µL	1x	
20x Fluorescent Dye	1.25µL	1x	
RTase Go	2.00µL	1x	
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 as necessary for low copy targets, challenging templates, or whenever amplification times have been reported to be slow.

   To improve sensitivity and specificity we recommend a quick cycle at the beginning of the reaction to facilitate the correct annealing of primers to the RNA template (for example 65˚C for 1 min, followed by a slow reduction of temperature to ambient value before starting the amplification).

   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