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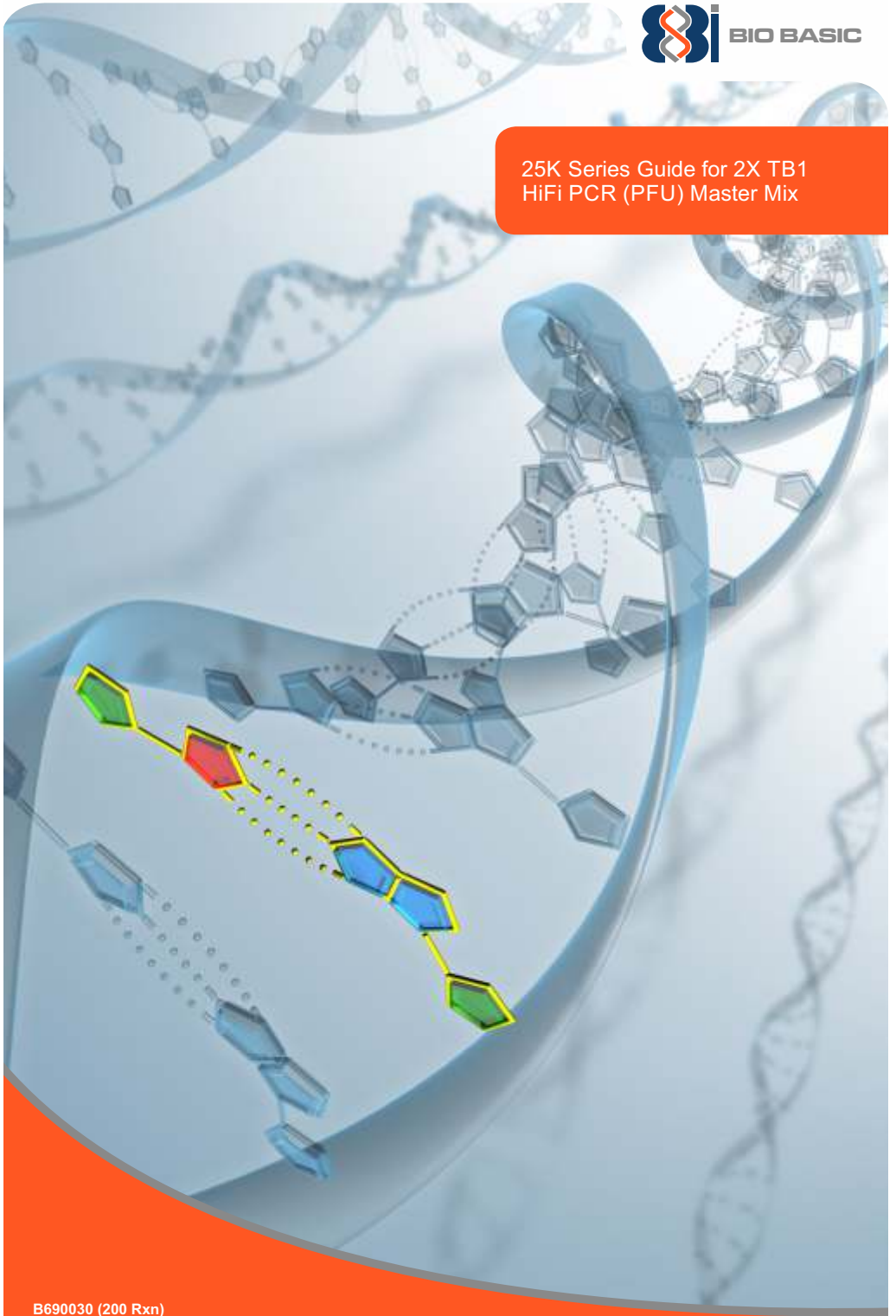
25K Series Guide for 2X TB1 HiFi PCR (PFU) Master Mix



B690030 (200 Rxn)
QF 24 TV4
Cv2 2025

For Research Use Only

25K Series Guide for 2X TB1 HiFi PCR (PFU) Master Mix

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Table of Materials

Contents for 25K Series for 2X TB1 HiFi PCR (PFU) Master Mix

Components	SKU	Volume	Rxn
2X TB1 HiFi PCR MM w/o Dye (PFU)	B690030	5000ul	200

Storage

Store at -20°C upon arrival. Transported at low temperature.

Intended Use

This product is for scientific research only and must not be used in medical or diagnostic procedures on humans or animals. It cannot be used as food, cosmetics, or household items. Without written permission or authorization, you may not manufacture, offer to sell, sell, import the product, or use any related patents or trademarks associated with the product. If you need additional usage permissions, please contact the manufacturer or visit their website. You must comply with all applicable licensing requirements listed on the product webpage when using this product. It is your responsibility to read, understand, and comply with all restrictive terms of these statements.

Quality Control

In accordance with Bio Basic ISO-certified Quality Management System, each lot of the 25K TB1 HiFi PCR (PFU) MasterMix is tested against predetermined specifications to ensure consistent product quality.

Introduction

25K TB1 HiFi PCR (PFU) MasterMix is an innovative, high-fidelity DNA polymerase complex designed for efficient, rapid, and reliable PCR amplification. I Its core advantage lies in its ultra fast extension rate, capable of amplifying most DNA fragments up to 10 kb at a speed of 5 seconds per kilobase (s/kb), significantly reducing experimental time. This enzyme system exhibits high fidelity suitable for cloning and sequencing applications, and demonstrates robust performance even with crude sample templates. The kit provides the enzyme and an optimized 2X reaction buffer as separate components, requiring the user to assemble the final reaction mix.

Source

Genetically engineered variant of Archael source DNA Polymerase that was purified from E. coli.

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nmol of dNTPs into acid insoluble material in 30 min at 70°C.

Standard PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions might vary (incubation times and temperatures, primers, Mg2+ and template DNA) and need to be optimized for each specific PCR. All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up and subsequent reaction(s) should be performed in separate areas to avoid cross-contamination. A negative control reaction (omitting template DNA) should always be performed in tandem with sample PCR to confirm the absence of DNA contamination.

Reaction Setup

Prepare the reaction mix in a 0.2mL PCR tube on ice as follows (for a 50 µL total volume):

Components	Volume per Rxn	Final Concentration
Template DNA*	X µL	See notes below
Forward Primer (10 µM)	2 µL	0.4 µM
Reverse Primer (10 µM)	2 µL	0.4 µM
2X TB1 HiFi PCR MM (PFU)	25 µL	1X
Nuclease-Free Water (ddH_2O)	Up to 50 µL	-
Components	**50 µL**	

*The recommended loading amount for templates such as genomic DNA and plasmids is 1-100 ng; for complex samples like cDNA, E. coli bacterial lysates, animal and plant cell extracts, and anticoagulated blood, the recommended loading amount is 0.5-5 µL; for solid templates such as single E. coli colonies, blood cards, and hair follicles, amplification is recommended using a sample the size of a toothpick tip or an area of ~1 mm^2.

Thermocycler Procedure

Mix contents of tube and centrifuge briefly. Run the following amplification procedure.

Place the PCR tube on the PCR instrument and run the following amplification procedure.

Step	Temperature	Time	Cycles	Notes
Initial Denaturation	98°C	30 s	1	Most templates
Cycling			**30-35**	
Denaturation Annealing Extension	98°C 60°C 72°C	10 s 5 s *See notes		Adjust based on primer
Final Extension Hold	72°C 12°C	2 min ∞	1 1	

Extension Time Recommendations

Length	Time
< 1 kb	1 s/kb
< 10 kb	5 s/kb
10 kb - 30 kb	5-10 s/kb
30 kb	10 s/kb

Troubleshooting

Long Fragments (>10 kb):

- Use high-quality template DNA.
- Design primers 25-35 nt long with Tm > 65°C.
- Consider reducing final primer concentration to 0.15 µM.
- For low sensitivity, try increasing final primer concentration up to 0.5 µM.

Note: Optimal performance up to 10 kb.

Complex Samples:

- Excessive input from reverse transcription reactions can inhibit PCR; ensure cDNA template amount (representing original RNA) is appropriate (e.g., < 750 ng input RNA equivalent per 50 µL PCR).
- Use EDTA or Sodium Citrate anticoagulant tubes for direct blood samples.
- If directly testing tissue, thoroughly crush and grind tissue samples for higher efficiency.

Non-Specific Amplification:

- If using a 3-step protocol yields non-specific bands, try a 2-step protocol (combine annealing/extension if primer Tm allows) or Touchdown PCR.
- Consider optimizing annealing temperature or slightly increasing extension time.