2X PCR BestaqTM MasterMix

Store at -20°C

Cat. No.	Description	Quantity
G464	2X PCR BestaqTM MasterMix	5 ml
G464-dye	2X PCR BestaqTM MasterMix with dye	5 ml

Product Description

crb's 2X PCR Bestaq™ MasterMix is a ready-to-use mixture containing **crb'**s Bestaq™ DNA Polymerase, dNTPs, and reaction buffer with proprietary additives in a 2X concentration. It contains all the necessary reagents for an efficient, high-yield amplification of DNA. The 2X PCR Bestaq™ MasterMix with dye (Cat. No. G464-dye) contains a green dye blend which resolves during gel electrophoresis into a turquoise band at ~4000 bp and a yellow band at the ~50 bp region. The resolving bands of dye allow easy visualization of the real time progress of your gel electrophoresis, and the yellow band indicates the migrating front in the gel.

crb's Bestaq™, with its superior fidelity and extreme robustness, is one of the best-performing DNA polymerases available on the market. With its monomeric, unique topological structure, Bestaq™ DNA Polymerase possesses enhanced processivity, elevated yield, boosted speed, extended amplification length, and improved difficult template tolerance. This high-performing polymerase and its MasterMix are the ideal choice for any PCR application, especially for cloning difficult templates, amplifying A/T- and G/C-rich sequences, and replicating long amplicons. With over 50X more accuracy than Taq DNA polymerase, Bestaq™ DNA Polymerase and its MasterMix can consolidate all PCR protocols and reactions into one simple, efficient, and extremely reliable PCR system. Bestaq™ DNA Polymerase generates blunt-end PCR products and has both 5'-3' polymerase activity and 3'-5' proofreading exonuclease activity.

Features and Benefits

- Saves preparation time by combining Bestaq™ DNA Polymerse, dNTPs and reaction buffer into a ready-to-use mixture.
- Reduces the risk of contamination by decreasing the number of pipetting steps.
- Provides consistent reaction performance and results.

Shipping and Storage

Keep at −20°C for long-term storage. 2X PCR Bestaq™ MasterMix and 2X PCR Bestaq™ Mas-terMix with dye are stable at 4°C for one month or for fifteen freeze-thaw cycles. For daily use, it is recommended to keep an aliquot at 4°C.

Protocol

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. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended. Always keep the control DNA and other templates to be amplified isolated from the other components.

A negative control reaction (omitting template DNA) should always be performed in tandem with sample PCR to confirm the absence of DNA contamination.

1. Add the following components to a sterile 0.2 ml PCR tube sitting on ice.

Components	Volume	Final Concentration
Template DNA	~100 ng	~2 ng/µl
Forward primer (10 µM)	1 - 2.5 µl	200 - 500 nM
Reverse primer (10 µM)	1 - 2.5 µl	200 - 500 nM
2X PCR Bestaq™ MasterMix/ with dye	25 µl	1X
Nuclease-free H_2O	up to 50 µl	-

- *We recommend preparing a mastermix for multiple reactions to minimize reagent loss and enable accurate pipetting.*

2. Mix contents of tube and centrifuge briefly.
3. Incubate tube in a thermal cycler at 94°C for 3 mins to completely denature the template.
4. Perform 30 - 35 cycles of PCR amplification as follows:
   Denature: 94°C for 10 secs
   Anneal: 45 - 72°C for 30 secs
   Extend: 72°C for 1 min/3 - 4 kb template
5. Incubate for an additional 5 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
6. Anlyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or SafeView™ (Cat No. G108) staining. If 2X PCR Bestaq™ MasterMix with dye is used, load the samples directly without adding additional loading dye. Use appropriate molecular weight standards.