2X PCR HotStart MasterMix

Store at -20°C

Cat. No.	Description	Quantity
G906	2X PCR HotStart MasterMix	5 ml
G906-dye	2X PCR Taq HotStart with dye	5 ml

Product Description

The 2X PCR HotStart MasterMix is a ready-to-use mixture of high quality HotStart DNA Polymerase, deoxynucleotides, and reaction buffer in a 2X concentration. It contains all the necessary reagents for amplification of DNA. HotStart DNA Polymerase contains a proprietary antibody that blocks polymerase activity at low temperatures. During the initial denaturation step at 94°C, the antibody dissociates from Taq DNA polymerase and restores enzyme activity. This feature significantly reduces non-specific product formations that would otherwise compete for reagent availability. Thus, **crb**'s HotStart DNA Polymerase offers improved yield of desired PCR products.

The 2X PCR HotStart MasterMix with dye contains, in addition, an inert green dye blend and a stabilizer which allows for direct loading of the final products onto a gel for analysis. The green dye blend resolves during gel electrophoresis into a turquoise band at ~4000bp and a yellow band at the ~50bp region. The resolving bands of dye allow easy visualization of the real time progress of your gel electrophoresis, where the yellow band indicates the migrating front in the gel.

To set up a PCR reaction: add DNA template, primers and water. PCR products that are amplified up to 6 kb in length with Taq DNA Polymerase contain a single base (A) 3' overhang.

Features and Benefits

- Saves preparation time by combining HotStart DNA Polymerse, dNTPs and reaction buffer in 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 HotStart MasterMix and 2X PCR HotStart MasterMix with dye are stable at 4°C for three months or for fifteen freeze-thaw cycles. For daily use, we recommend keeping 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", automatic 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 HotStart 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 10 mins to completely activate the HotStart DNA Polymerase and denature the template.
4. Perform 30 - 35 cycles of PCR amplification as follows:

   Denature: 94°C for 30 sec

   Anneal: 45 - 72°C for 30 sec

   Extend: 72°C for 1 min/1 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. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or SafeView™ (Cat No. G108) staining. If 2X PCR HotStart MasterMix with dye is used, load the samples directly without adding additional loading dye. Use appropriate molecular weight standards.