

## 2X PCR Taq Plus MasterMix

Store at -20°C

Cat. No.	Description	Quantity
G014	2X PCR Taq Plus MasterMix	5 ml
G014-dye	2X PCR Taq Plus MasterMix with dye	5 ml

### Product Description

**crb's** 2X PCR Taq Plus MasterMix is a ready-to-use mixture of high-quality Taq Plus DNA Polymerase, deoxynucleotides, and reaction buffer in a 2X concentration. It contains all the necessary reagents for amplification of DNA. The 2X PCR Taq Plus MasterMix with dye contains an inert blue dye and a stabilizer which allow direct loading of the final products onto a gel for analysis.

To set up a PCR reaction: add DNA template, primers and water. PCR products, amplified up to 6 kb in length with Taq Plus DNA Polymerase, contain a mixture of blunt ends and single base (A) 3' overhang. The error rate of this PCR amplification is  $7.5 \times 10^{-6}$  per nucleotide per cycle. The products can be used for direct T/A cloning, but its efficiency is not as high as PCR products amplified with Taq DNA Polymerase alone.

### Features and Benefits

- Saves preparation time by combining Taq Plus DNA Polymerase, 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 Taq Plus MasterMix and 2X PCR Taq Plus 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" 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/ $\mu$ l
Forward primer (10 $\mu$ M)	1 - 2.5 $\mu$ l	200 - 500 nM
Reverse primer (10 $\mu$ M)	1 - 2.5 $\mu$ l	200 - 500 nM
2X PCR Taq Plus MasterMix/ with dye	25 $\mu$ l	1X
Nuclease-free H <sub>2</sub> O	up to 50 $\mu$ l	-

- 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 30 secs
    - Anneal:** 45 - 72°C for 30 secs
    - 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 Taq Plus Mastermix with dye is used, load the samples directly without adding additional loading dye. Use appropriate molecular weight standards.