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CAUTION! Professional use only.
Follow instruction manual before use.
Reading and carefully following these warnings will minimize usage errors, other possible risks and hazards.
Wear safety glasses, clothes, and gloves.

# 2X PCR MASTER MIX

User Guide

| Catalog Number | Pack Size |
|---|---|
| ZT-PCRMX-1 | 1,25ML (100RXN) |
| ZT-PCRMX-5 | 5 X 1,25ML (5*100RXN) |

## Content and Storage Condition

| Content | Quantitiy | Transportation Condition | Storage Condition |
|---|---|---|---|
| 2X PCR MASTER MIX | 1,25ML | -20°C | -20°C |

**Features**

**Concentration:** 2X

**Application:** Molecular Biology

**Purity Class:** Molecular Biology

**Appearance:** Colorless, clear, liquid

**Classification:** General Substance

**Reaction Speed:** Standard

**Polymerase:** Taq Polymerase

### 2X PCR Master Mix Composition

0.05 U/µL Taq DNA polymerase, 10X Reaction buffer, 4 mM MgCl2, 0.4mM each dATP, dCTP, dGTP and dTTP
It is a ready-to-use mixture.
Its half-life is more than 40 minutes at 95 ° C.
Produces 3'-dA PCR products
Contains modified nucleotides (for example, biotin-, digoxigenin-, fluorescently labeled nucleotides)

### Applications

Routine PCR amplification of DNA fragments up to 5 kb in length and high throughput PCR.

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## Product Description

SOFTEC 2X PCR Master Mix is a 2X concentration mixture consisting of all the components required for PCR except DNA template and primers. This pre-mixed formulation saves time and reduces contamination due to the reduction of pipetting steps required for PCR setup. The mixture is optimized for efficient and reproducible PCR. It is thermostable. Its half-life is more than 40 minutes at 95 ° C.

## Note

The error rate of Taq DNA Polymerase in PCR is 2.2 x 10-5 errors per nucleotide in each cycle. The accuracy of PCR is 4.5 x 104. Accuracy is the inverse of the error rate and refers to the average number of correct nucleotides included before an error occurs.

## Protocol

1. Gently vortex and briefly centrifuge SOFTEC 2X PCR Master Mix after thawing on ice.
2. Place your 0.2ml microcentrifuge tubes on the ice and follow the table below for each 25 µL reaction volume.

| | |
|---|---|
| SOFTEC 2X PCR Master Mix | 12,5 µL |
| Forward Primer | 0,5 µL (10µM) |
| Reverse Primer | 0,5 µL (10µM) |
| Template DNA | 10pg - 1µg |
| Nuclease free water | to 25µL |

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Perform PCR by following the table below for recommended thermal cycling conditions:

| **Step** | **Temperature (°C)** | **Time** | **Number of cycle** |
|---|---|---|---|
| Pre-denaturation | 95 | 1-3 min | 1 |
| Denaturation | 95 | 30 s | 25-40 |
| Annealing | Tm-5 | 30 s | |
| Extension | 72 | 1 min /1kb | |
| Final extension | 72 | 5-15 min | 1 |

## Recommendations for Preventing Contamination for the PCR Reaction

- Perform the preparation of the DNA sample, preparation of the PCR mixture, use of the
- thermal cycling device and analysis operations in separate areas.
- Perform the preparation of the PCR mixture in a laminar flow cabinet with a UV lamp.
- Wear clean laboratory gloves and renew your gloves in different steps.
- Always perform "no template control" (NTC) reactions to check for contamination.

## Tips

For GC-rich DNA templates, DNA denaturation time of 30 seconds can be prolonged to 3-4 min. Primer annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers.

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