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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 Superstrong PCR Master Mix (Ready-to-load)

User Guide

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

Content and Storage Condition

Content	Quantitiy	Transportation Condition	Storage Condition
2X SUPERSTRONG PCR MASTER MIX (Ready-to-load)	1,25ML	-20°C	-20°C

Features

Concentration: 2X

Application: Molecular Biology

Purity Class: Molecular Biology

Appearance: Light Blue, liquid

Classification: General Substance

Reaction Speed: Standard

Polymerase: Taq Polymerase

Benefit:

i) No loading buffer/tracking dye needs to be added. The PCR product is loaded directly into an agarose gel after amplification.
ii) Ready-mix format reduces setup time.
It is a ready-to-use and ready-to-load 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)

2X SuperStrong PCR Master Mix (Ready-to-load) Composition

0.05 U/µL Taq DNA polymerase, 10X Reaction buffer, 4 mM MgCl2, 0.4mM each dATP, dCTP, dGTP, dTTP and inert blue dye in a 2X concentrate.

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Applications

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

Product Description

SOFTEC 2X SUPERSTRONG PCR Master Mix (Ready-to-load) 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.

After the PCR reaction, the PCR product can be loaded directly onto an agarose gel. The blue loading dye migrates in an agarose gel. The inert dye has no effect on the amplification process, and therefore, a sample can be easily re-amplified such as in "nested PCR".

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 SUPERSTRONG PCR Master Mix (Ready-to-load) 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 (Ready-to-load)	12,5 µL
Forward Primer (10µM)	0,5 µL – 2 µL
Reverse Primer (10µM)	0,5 µL – 2 µL
Template DNA	10 pg – 1 µg
Nuclease free water	Up 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 12,5 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.
Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.
The recommended extension step is 1 min/kb at 72° for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 1 min/kb.
For less than 10 copies of the template in the reaction, 40 cycles are recommended.
For higher template amounts, 25-35 cycles are sufficient.

Warning

Do not take delivery of the product whose packaging is damaged and do not use it.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay: No detectable degradation of DNA was observed after incubation of 1 µg of pUC19 DNA with 25 µL of SOFTEC 2X SUPERSTRONG PCR Master Mix (Ready-to-load) in 50 µL of reaction mixture for 4 hours at 37°C and at 70°C.
Exodeoxyribonuclease Assay: No detectable degradation of DNA was observed after incubation of 1 µg of lambda DNA/HindIII fragments with 25 µL of SOFTEC 2X SUPERSTRONG PCR Master Mix (Ready-to-load) in 50 µL of reaction mixture for 4 hours at 37°C and at 70°C.
Ribonuclease Assay: No contaminating RNase activity was detected after incubation of 1 µg of [3H]-RNA with 25 µL SOFTEC 2X SUPERSTRONG PCR Master Mix (Ready-to-load) in 50 µL of reaction mixture for 4 hours at 37°C and at 70°C.

Quality authorized by: Zet Medical R&D Lab