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PCR Master Mix 2x

#AUPREP-RMX-2X 1ml, 100rxn of 20µl

Contents: 2X Taq Ready Mix 1ml

Nuclease-free water 1ml

Store at -20°C

Shelf life: 1 year from date of shipping

Description

PCR Ready Mix (2x) is a premixed, ready-to-use solution containing Taq DNA Polymerase, dNTPs, Mg2+ and Reaction Buffer at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only primers and template DNA are added. This pre-mixed formulation saves time and reduces contamination due to the fewer pipetting steps required for PCR set up. The mix retains all features of Taq DNA Polymerase. Taq DNA Polymerase is a thermostable recombinant DNA polymerase derived from thermophilic bacterium *Thermus aquaticus.* Its molecular weight is 94 kDa. Taq DNA Polymerase can amplify DNA target up to 5 kb (simple template). The elongation velocity is 0.9~1.2kb/min (70~75°C). It has 5' to 3' polymerase activity but lacks of 3' to 5' exonuclease activity that results in a 3'-dA overhangs PCR product.

Applications

- High throughput PCR.
- Routine PCR with high reproducibility
- Generation of PCR products for TA cloning

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Feature

- Convenient–Taq DNA Polymerase in a ready-to-use mixture.
- High yields of PCR products with minimal optimization.
- Fast -saves time due to reduced number of pipetting steps.
- Reproducible -lower contamination and pipetting error risk.

Composition of the 2X Taq Ready Mix

Taq DNA polymerase is supplied in 2X Taq buffer, dNTPs, 3mM MgSO4 and bromophenol blue. Taq Ready mix buffer is a proprietary formulation optimized for robust performance in PCR.

Protocol

All solutions should be thawed on ice, gently vortexed and briefly centrifuged.

1. **Add in a thin walled PCR tube on ice:**

   For a total 20µl reaction volume:

Component of Sample	Volume	Final concentration
Taq Ready Mix (2X)	10 µl	1X
Forward Primer	variable	0.1-1µM
Reverse Primer	variable	0.1-1µM
Template DNA	variable	10 pg-1µg
Water, nuclease-free	to 20µl	—

Recommendations with Template DNA in a 20µl reaction volume:

Human genomic DNA	0.1 µg-1 µg
Plasmid DNA	0.2 ng-3 ng

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Phage DNA	0.1 ng-4 ng
E.coli genomic DNA	4 ng-40 ng

2. Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube.

3. Overlay the sample with mineral oil or add an appropriate amount of wax. This step may be omitted if the thermal cycler is equipped with a heated lid.

4. Perform PCR using the following thermal cycling conditions.

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1 min
Final Extension	72°C	10 minutes

Guidelines for preventing contamination of PCR reaction:

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination.

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Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests, functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 25µl PCR Ready Mix (2X) with 1 µg of pBR322 DNA in 50µl for 4 hours at 37°C and at 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 25µl of PCR Ready Mix (2X) with 1µg of digested DNA in 50µl for 4 hours at 37°C and at 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25µl of PCR Ready Mix (2X) with 1µg of E.coli [3H]-RNA (40000cpm/µg) in 50µl for 4 hours at 37°C.

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25 µl of Taq Ready Mix (2X) with 1µg of E.coli [3H]-RNA (40000cpm/µg) in 50µl for 4hours at 70°C.

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