PCR mastermix

2X PCR MasterMix (Dye Plus)	1 ml

For research use only
Cat No: YT1551
Size: 1 ml
Store at –20°C

Introduction

2× PCR mastermix 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 need to add primers and template DNA. This premixed 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.

Composition :

0.25U/ul Taq DNA Polymerase, PCR Buffer, 0.4mM dNTPs, MgCl2, bromophenol blue.
2x PCR buffer is a proprietary formulation optimized for robust performance in PCR.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitrouse only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Applications

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

Features

- Convenient: only primers and template DNA are added when prepare final PCR
- High yields of PCR products with minimal optimization
- High efficiency: saving your time by simplifying the process
- Reproducible: lower contamination and pipetting error risk

Basic PCR Protocol

All solutions should be thawed on ice, gently vortex and briefly centrifuge

1. **Add the following components to a sterile micro centrifuge tube sitting on ice:**

Reagents	Quantity	Final concentration
2x PCR mastermix	12.5 µl	1X
Forward primer	variable	0.4-1 µM
Reverse Primer	variable	0.4-1 µM
Template DNA	variable	10pg- 1 µg
Nuclease free water	To 25 µl	-

***Recommended amount of DNA template for a 50 µl reaction system is as follow:**

Human genomic DNA	0.1～1 µg
plasmid DNA	0.5～5 ng
phage DNA	0.1～10 ng
E.coli genomic DNA	10～100ng

2. Mix contents in the tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.
When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl mineral oil.
3. Perform 25-35 cycles of PCR amplification as follows:

Initial denaturation	94 ˚ C	3 min
25-35 Cycles	94 ˚ C 55 68 ˚ C 72 ˚ C	30 sec 30 sec 1-10 min
Final extension	72 ˚ C	10 min

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
5. Analyze the amplification products by agarose gel electrophoresis and visualize by nucleic acid dye staining. Use appropriate molecular weight standards.
Notes on cycling conditions
• Recombinant Taq DNA Polymerase is suitable for most PCR applications.
• The half-life of Taq DNA Polymerase is >40 minutes at 95°C.
• The error rate of Taq DNA Polymerase in PCR is 2.2×10-5 errors per nt per cycle
• Taq DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
• The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reactions. Low amounts of starting template may require 40 cycles.