Taq Mix

Catalog No: P1051 (1 ml)

Contents:	2X Taq Mix	1ml
	Nuclease-free water	1ml

Description

2X Taq Mix is a premixed, ready-to-use solution containing Taq DNA Polymerase, dNTPs, Mg^{2+} 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 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

PRODUCT USE LIMITATION.

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

Feature

- **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.

Composition of the 2xTaq Mix

0.25U/ul Taq DNA polymerase, 2X PCR buffer, 0.4mM dNTPs, 3.2 mM $MgCl_2$, 0.02% bromophenol blue.

Taq mix buffer is a proprietary formulation optimized for robust performance in PCR.

Basic 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 50µl reaction volume

Component of sample	Volume	Final concentration
Taq Mix (2X)	25 µl	1X
Forward Primer	variable	0.1-1 µM
Reverse Primer	variable	0.1-1 µM
Template DNA	variable	10 pg-1 µg
Water, nuclease-free	to 50 µl	–

Recommendations with Template DNA in a 50µl reaction volume

Human genomic DNA	0.1 µg-1 µg
Plasmid DNA	0.5 ng-5 ng
Phage DNA	0.1 ng-10 ng
E.coli genomic DNA	10 ng-100 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. Preform 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 minutes
Final Extension	72°C	10 minutes

5. 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.

6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

Notes on cycling conditions

- Recombinant Taq DNA Polymerase is the enzyme of choice for most PCR applications.
- The half-life of enzyme 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; the accuracy (an inverse of the error rate) an average number of correct nucleotides incorporated before making an error, is 4.5×10^{-4} (determined according to the modified method described in).
- 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 enough for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

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

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases are 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 Taq Mix (2X) with 1µg pBR322 DNA in 50µl for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

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

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25µl Taq Mix (2X) with 1µg E. coli [3H]-RNA (40000cpm/µg) in 50µl for 4 hours at 37°C and 70°C.