# Taq Mix (2x)

**Ref: NB-03-0088 1ml**
**NB-03-0089 1ml x 5**

## Contents

| **NB-03-0088** | |
|---|---|
| 2×PCR Taq Mix | 1 ml |
| Nuclease-Free Water | 1 ml |

**Note** : The system contains sufficient reagents suitable for 40 amplification reactions of 50 µl each.

| **NB-03-0089** | |
|---|---|
| 2×PCR Taq Mix | 1 ml×5 |
| Nuclease-Free Water | 1 ml×5 |

**Note** : The system contains sufficient reagents suitable for 200 amplification reactions of 50 µl each.

## Description

2X Taq Mix 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

## Features

• Convenient –Taq DNA Polymerase in a ready-to-use Mix.

• 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.

## Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nM of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

## Composition of the Taq Mix

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

## Store at -20°C

## 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 | 100 ng – 100 ng |

## 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

| Components | Volume | Final Concentration |
|---|---|---|
| 2×PCR Taq Mix | 25 µl | 1 × |
| Primer mix (10 µM each) | Variable | 0,4 - 1µM |
| Template DNA | variable | 10 pg - 1µg |
| Nuclease-Free Water | to 50 µl | |

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.

| Step | Temperature | Duration |
|---|---|---|
| Initial denaturation | 94℃ | 3 min |
| | 94℃ | 30 s |
| 25-35 cycles | 55-68°C | 30 s |
| | 72°C | 1 min |
| Final extension | 72℃ | 10 min |

5. Maintain the reaction at 4°C. The samples can be stored at -20°C until use.

**Notes on cycling conditions :**

- Initial denaturation can be performed over an interval of 1-5 min at 95℃ depending on the GC content of template.
- Optimal annealing temperature is 5℃ lower than the melting temperature of primer-temperature DNA duplex. If nonspecific PCR products are obtained optimization of annealing temperature can be performed by increasing temperature stepwise by 1-2℃.
- 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 reaction. Low amounts of starting template may require 40 cycles.
- The time of the final extension step can be extended for amplicons that will be cloned into T/A vectors.

## *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 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 25 µl Taq Mix (2X) with 1 µg of pBR322 DNA in 50 µl 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 25 µl of Taq Mix (2X) with 1 µg of digested DNA in 50 µl 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 25 µl of Taq Mix (2X) with 1 µg of E.coli [3H]-RNA (40000cpm/µg) in 50 µl for 4hours at 37°C.