

# Pfu Mix

## #P2021, 1 ml

### Contents:

|                     |      |
|---------------------|------|
| 2× Pfu Mix          | 1 ml |
| Nuclease-free water | 1 ml |

Store at -20°C

For research use only.

In total 2 vials.

### Description

2× Pfu Mix is a premixed, ready-to-use solution containing hotstart Pfu DNA Polymerase with antibody modification, dNTPs, Mg<sup>2+</sup> 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. It also contributes to higher sensitivity by adding enhancer.

Pfu DNA Polymerase, derived from the hyperthermophilic archae *Pyrococcus furiosus*, has been shown to exhibit superior thermostability and proofreading properties compared to other thermostable polymerase. Unlike Taq DNA Polymerase, highly thermostable Pfu DNA Polymerase possesses 3' to 5' exonuclease proofreading activity that enables the polymerase to correct nucleotide-misincorporation errors. This means that Pfu DNA Polymerase-generated PCR fragments will have fewer errors than Taq-generated PCR inserts. Using Pfu DNA Polymerase in your PCR reactions results in blunt-ended PCR products, which are ideal for cloning into blunt-ended vectors. Pfu DNA Polymerase is superior for techniques that require high-fidelity DNA synthesis. The elongation velocity is 1kb/min(70~75°C).

### Composition of the 2× Pfu Mix

0.15U/μl Pfu DNA Polymerase, 2× Pfu Buffer, 0.4mM dNTPs, 4mM MgSO<sub>4</sub>, 0.02% bromophenol blue.

Pfu Mix buffer is a proprietary formulation optimized for robust performance in PCR.

## Applications

- High fidelity PCR
- Routine PCR with high reproducibility
- Site-directed mutagenesis
- 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
- **Fast:** saves time due to reduced number of pipetting steps.
- **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 microcentrifuge tube sitting on ice or at room temperature:**

| Reagent              | Quantity | Final concentration |
|----------------------|----------|---------------------|
| 2× Pfu Mix           | 25 µl    | 1×                  |
| Forward Primer       | variable | 0.4-1 µM            |
| Reverse Primer       | variable | 0.4-1 µM            |
| Template DNA         | variable | 10pg-1µg            |
| Water, nuclease-free | to 50 µl | –                   |

**Recommendation amounts of template DNA in a 50 µl reaction mix:**

|                    |            |
|--------------------|------------|
| Human genomic DNA  | 0.1µg-1µg  |
| Plasmid DNA        | 0.5ng-5ng  |
| Phage DNA          | 0.1ng-10ng |
| E.coli genomic DNA | 10ng-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 minutes                 |
| 25-35 Cycles         | 94°C            | 30 seconds                |
|                      | 55-68°C<br>72°C | 30 seconds<br>1-3 minutes |
| Final Extension      | 72°C            | 10 minutes                |

**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

- Pfu Mix is for high fidelity PCR applications.
- The error rate of Pfu DNA Polymerase in PCR is  $2.6 \times 10^{-6}$  errors per nt per cycle.
- Pfu DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The enzyme has no detectable reverse transcriptase activity.
- 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.

### ***Guidelines for preventing contamination of PCR***

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  $\mu$ l Pfu Mix (2 $\times$ ) with 1 $\mu$ g pBR322 DNA for 4 hours at 37°C and 70°C.

### ***Exodeoxyribonuclease Assay***

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 25  $\mu$ l Pfu Mix (2 $\times$ ) with 1 $\mu$ g digested DNA 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 25  $\mu$ l Pfu Mix (2 $\times$ ) with 1 $\mu$ g E.coli [3H]-RNA (40000cpm/ $\mu$ g) for 4 hours at 37°C and 70°C.

### **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.