

Pfu Kit

#P3022, 5 ml

Contents:

2× Pfu Reaction Mix	1 ml × 5
Pfu DNA Polymerase (2.5U/μl)	160 μl

Store at -20°C

For research use only.

In total 6 vials.

Description

Pfu Kit contains Pfu DNA Polymerase and a premixed, ready-to-use Pfu Reaction Mix, which contains dNTPs, Mg^{2+} and other components at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only need to add primers and template DNA. The reaction mix contributes to high specificity by optimizing the system, reducing primer-dimer rate. PCR amplification sensitivity is controllable due to the amount of DNA polymerase is flexible.

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)

Unit Definition

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

Storage Buffer

20mM Tris-HCl (pH8.0), 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.5% NP-40, 0.5% TW 20, 50% Glycerol

Composition of the 2× Pfu Reaction Mix

2× Pfu Buffer, 0.4mM dNTPs, 3.2mM MgSO₄, 0.02% bromophenol blue.

Pfu Reaction Mix 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
- **Flexible:** the amount of polymerase is flexible and controllable.

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:

Reagent	Quantity	Final concentration
2× Pfu Reaction Mix	25 µl	1×
Forward Primer	variable	0.4-1µM
Reverse Primer	variable	0.4-1µM
Template DNA	variable	10pg-1µg
Pfu DNA Polymerase (2.5U/µl)	0.5-1 µl	1.25-2.5U/50µl
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
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25-35 Cycles	94°C	30 seconds
	55-68°C	30 seconds
	72°C	1-5 minutes
Final Extension	72°C	2 minutes

4. Incubate for an additional 2 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

- The optimal reaction conditions (incubation time and temperature, concentration of Pfu DNA Polymerase, template DNA, MgSO₄) depend on the template-primer pair and must be determined individually. It is especially important to titrate the MgSO₄ concentration and the amount of enzyme required per assay. The standard concentration of MgSO₄ is 2mM and amount of Pfu DNA Polymerase is 1.25u per 50µl of reaction mixture.
- Pfu DNA Polymerase remains 95% active after 2 hours incubation at 95°C.
- The error rate of Pfu DNA Polymerase in PCR is 2.6x10⁻⁶ 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 10U Pfu DNA Polymerase with 1µg 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 10U Pfu DNA Polymerase with 1µg 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 10U Pfu DNA Polymerase with 1µg E.coli [3H]-RNA (40000cpm/µg) 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.