Pfu Kit

Catalog No.: P2011 (5x1ml)

Contents:	2X Pfu Reaction Mix	5x1ml
	Pfu DNA Polymerase (2.5U/µl)	160µl

Store at -20°C

In total 6 vials

Description

Pfu Kit contains a pfu DNA polymerase and a 2x premixed, ready-to-use pfu reaction Mix. The pfu reaction mix is supplied as a 2X concentrated solution, that contains dNTPs and all other PCR components, except DNA template and primers. To prepare the final PCR reaction system, mix pfu DNA polymerase and the pfu reaction mix, then add primer and template. 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. Pfu DNA polymerase is superior for techniques that require high-fidelity DNA synthesis.

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.

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

20 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5 % TW 20, 0.5 % NP 40, 50 % Glycerol

Composition of 2X Pfu Reaction Mix

2xPfu Buffer, 0.4mM dNTPs, 4mM $MgSO_4$, 0.02% bromophenol blue.
2xPfu Reaction mix is a proprietary formulation optimized for robust performance in PCR.

Features

·**Convenient:** only primers and template are needed

·**Efficiency:** simplifying the operation and saving your time

·**Reproducible:** reduce the risk of pipetting errors, miscalculation and contamination.

·**Flexible:** the amount of polymerase is flexible and controllable.

Applications

·PCR amplification of DNA fragments as long as 5 kb

·DNA labeling.

·DNA sequencing.

·PCR for cloning

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
2X Pfu Reaction Mix	25 µl	1X
Forward Primer	variable	0.1-1 µM
Reverse Primer	variable	0.1-1 µM
Template DNA	variable	10 pg-1 µg
Pfu Taq DNA Polymerase (2.5U/µl)	0.5-1 µl	1.25-2.5U/50µl
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 g-100 ng

2. Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube. 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.

3. Place samples in a thermocycler and start the program
Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 2-3 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 ethidium bromide 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, $MgSO_4$) depend on the template-primer pair and must be determined individually. It is especially important to titrate the $MgSO_4$ concentration and the amount of enzyme required per assay. The standard concentration of $MgSO_4$ 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 ncubation at 95°C.
- The error rate of Pfu DNA Polymerase in PCR is $2.6x10^{-6}$ errors per nt per cycle; the accuracy (an inverse of error rate) an average number of correct nucleotides incorporated before making an error is $3.8x10^{-5}$ (determined according to the modified method described in).
- 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.
- Do not use dUTP in PCR.
- 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 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:

a. Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
b. Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
c. Wear fresh gloves for DNA purification and reaction set up.
d. 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.
e. Always perform "no template control" (NTC) reactions to check for contamination.

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonu- cleases 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.