PCR Kit

Catalog No: P1041 (5x1 ml)

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

Description

The PCR Kit contains a supply of recombinant, highly purified Taq DNA Polymerase, and a premixed, ready-to-use 2x PCR Reaction Mix to perform 200 PCR reactions.

2x PCR Reaction Mix is a premixed, ready-to-use solution containing dNTPs and reaction buffer at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, please mix the polymerase and the reaction buffer in appropriate proportion, and then add the primers and template DNA. PCR Kit contributes to highly reproducible PCR by reducing the risk of pipetting errors, miscalculation and contamination. It also contributes to higher sensitivity by adding enhancer. Using the kit in your PCR reaction results in 3'-dA overhangs PCR products.

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 herring sperm DNA as substrate.

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.

Storage Buffer

20mM TrisCl (pH 8.0), 100mM KCl, 3.2mM $MgCl_2$ 1mM DTT, 0.1% Triton X-100 ,0.1% Tween20, 0.2mg/ml BSA, 50% (v/v) glycerol.

Composition of 2X PCR Reaction Mix

2X PCR buffer, 0.4mM dNTPs, 3.2 mM $MgCl_2$, 0.02% bromophenol blue.

PCR Reaction Mix is a proprietary formulation optimized for robust performance in PCR

Features

- **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 PCR 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
Taq DNA Polymerase (2.5U/µl)	0.5-1µl	1.25-2.5U/50µl
Water, nuclease-free	to 50µl	–

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. 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 1 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

- 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 $2.2x10^{-5}$ 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 $4.5x10^{-4}$ (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 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:

- 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 Taq 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 Taq 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 Taq DNA Polymerase with 1µg E. coli [3H]-RNA (40000cpm/µg) for 4 hours