G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Pfu DNA Polymerase Mastermix [2X]

(Cat. # 786-817)

INTRODUCTION

Pfu DNA Polymerase Mastermix [2X] is a premixed, ready-to-use solution containing Pfu DNA Polymerase, dNTPs, $MgSO_4$ 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. Pfu Mix contributes to highly reproducible PCR by reducing the risk of pipetting errors, miscalculation and contamination.

Pfu DNA polymerase, derived from the hyperthermophilic archae *Pyrococcus furiosus*, has been shown to exhibit superior thermostability and proofreading properties compared to other polymerases. Unlike Taq 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. Use Pfu polymerase for techniques that require high-fidelity DNA synthesis.

ITEM(S) SUPPLIED (Cat. # 786-817)

Description	Size
Pfu DNA Polymerase Mastermix [2X]	1ml
Water, Nuclease free	1ml

STORAGE CONDITIONS

It is shipped on blue ice. Upon arrival, store at -20°C. Pfu Polymerase 2X Mastermix is stable at 4°C for three months or fifteen freeze-thaws. For daily use we recommend storing at 4°C.

UNIT DEFINITION

One unit (U) of Pfu polymerase is defined as the amount of enzyme needed to catalyze the incorporation of 10 nanomoles of deoxyribonucleotides into acid-insoluble material in 30 minutes at 70°C using herring sperm DNA as a substrate.

PFU MIX COMPOSITION

Pfu DNA polymerase is supplied in 2X Pfu buffer, dNTPs, 3mM $MgSO_4$ and bromophenol blue. Pfu mix buffer is a proprietary formulation optimized for robust performance in 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
Pfu Mix (2X)	25µl	1X
Forward Primer	variable	0.1-1µM
Reverse Primer	variable	0.1-1µM
Template DNA	variable	10 pg-1µg
Water, nuclease-free	to 50µl	–

Recommendations with Template DNA in a 50µl reaction volume

Human genomic DNA	0.1µg-1µg
Plasmid DNA	0.5ng-5ng
Phage DNA	0.1ng-10ng
E.coli genomic DNA	10ng-100ng

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. Perform PCR using the following thermal cycling conditions.

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1 min
Final Extension	72°C	10 minutes

5. 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.
6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

GENERAL GUIDELINES

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 25µl Pfu Mix (2X) with 1µg of pBR322 DNA in 50µl for 4 hours at 37°C and at 70°C.

Exodeoxyribonuclease Assay

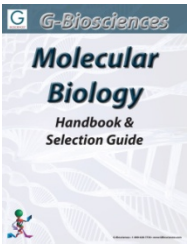
No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 25µl of Pfu Mix (2X) with 1µg of digested DNA in 50µl for 4 hours at 37°C and at 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25µl of Pfu Mix (2X) with 1µg of E. coli [3H]-RNA (40000cpm/µg) in 50µl for 4 hours at 37°C. 0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25µl of Pfu Mix (2X) with 1µg of E. coli [3H]-RNA (40000 cpm/µg) in 50µl for 4 hours at 70°C.

RELATED PRODUCTS

Download our Sample Preparation and Protein Purification Handbooks.



http://info.gbiosciences.com/complete-molecular-biology-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 5/19/2015 CMH

This page is intentionally left blank

www.GBiosciences.com