NeoBiotech

Pfu Mix (2x) for High Fidelity PCR

Cat # NB-03-0095 1ml

Cat # NB-03-0096 5 x 1ml

Description

2XPfu Mix is a premixed, ready-to-use solution containing Pfu DNA Polymerase, dNTPs, MgSO4 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. It also contributes to higher sensitivity by adding intensifier and optimizer.

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.

Contents

NB-03-0095	2×PCR Pfu Mix	1 ml
	Nuclease-Free Water	1 ml
	for 40 amplification reactions of 50 µl each	
NB-03-0096	2×PCR Pfu Mix	5 x 1 ml
	Nuclease-Free Water	5 x 1 ml
	for 200 amplification reactions of 50 µl each	

0.15U/ul Pfu DNA polymerase, 2xPfu Buffer, 0.4mM dNTPs, 4mM MgSO4, 0.02% bromophenol blue

Applications

High fidelity PCR
High reproducible PCR
PCR cloning
Site-directed mutagenesis

Unit Definition

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

Storage

Store at -20°C

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Basic PCR Protocol

1. Add the following components to a sterile 50 µl microcentrifuge tube sitting on ice:

Component of sample	Volume	Final concentration
Pfu Mix (2X)	25 µl	1X
Forward Primer	variable	0.1-1 µM
Reverse Primer	variable	0.1-1 µM
Template DNA	variable	10 pg-1 µg
Water, nuclease-free	to 50 µl	–

2. Mix contents of tube and overlay with 50 µl of mineral or silicone oil.
3. Cap tubes and centrifuge briefly to collect the contents to the bottom.
4. Incubate tubes in a thermal cycler at 94°C for 3 minutes to completely denature the template.
5. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94℃	3 minutes
25-35 Cycles	94℃ 55-68℃ 72℃	30 seconds 30 seconds 2-3 minutes
Final Extension	72℃	10 minutes

6. 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.

Notes on cycling conditions

a. Initial denaturation can be performed over an interval of 1-5 min at 95°C depending on the GC content of template.
b. Optimal annealing temperature is 5°C lower than the melting temperature of primer-temperature DNA duplex. If nonspecific PCR products are obtained optimization of annealing temperature can be performed by increasing temperature stepwise by 1-2°C.
c. 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.
d. The time of the final extension step can be extended for amplicons that will be cloned into T/A vectors.

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

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