Pfu DNA Polymerase

Ref : NB-03-0091 250 U
NB-03-0092 250 U
NB-03-0093 1 000 U
NB-03-0094 1 000 U

Contents

NB-03-0091	
Pfu DNA Polymerase (2.5 U/µl)	100 µl
10X Pfu Buffer (Mg^{2+} Plus)	1.4 ml
6X Loading Buffer	1 ml

NB-03-0092*	
Pfu DNA Polymerase (2.5 U/µl)	100 µl
10X Pfu Buffer (Mg^{2+} Plus)	1.4 ml
dNTPs (each 2.5 mM)	1 ml
6X Loading Buffer	1 ml

NB-03-0093	
Pfu DNA Polymerase (2.5 U/µl)	400 µl
10X Pfu Buffer (Mg^{2+} Plus)	1.4 ml x2
6X Loading Buffer	1 ml

NB-03-0094*	
Pfu DNA Polymerase (2.5 U/µl)	400 µl
10X Pfu Buffer (Mg^{2+} Plus)	1.4 ml x2
dNTPs (each 2.5 mM)	1 ml x2
6X Loading Buffer	1 ml

* with dNTPs mixture

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Description

Pfu DNA polymerase, derived from the hyperthermophilic archae *Pyrococcus furiosus*, has superior thermostability and proofreading properties compared to other thermostable polymerase. Its molecular weight is 90 kDa. It can amplify DNA target up to 2 kb (simple template). The elongation velocity is 0.2~0.4 kb/min (70~75°C).

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.

Features

- 3'-5' exonuclease activity provides a low error rate
- One of the most thermostable DNA polymerases known
- Lack of extendase activity means no unwanted 3' overhangs
- Optimal for blunt-ended PCR cloning
- Optimum temperature near 75°C
- 95% active after 1hour incubation at 98°C

Applications

- High-fidelity PCR and primer-extension reactions
- High fidelity PCR for cloning into blunt-ended vectors
- 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 Buffer

20mM TrisCl (pH 8.0), 100mM KCl, 3mM MgCl2 1mM DTT, 0.1% NP-40, 0.1% Tween20, 0.2mg/ml BSA, 50% (v/v) glycerol

10X Pfu Buffer with $MgSO_4$

200mM Tris-HCl(pH8.8 ， 25℃), 100mM KCl, 100mM (NH4)2SO4, 20mM MgSO4, 1.0% Triton®X-100, 1mg/ml BSA

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of Pfu DNA Polymerase, primers, Mg^{2+} and template DNA) vary and need to be optimized.

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

Reagent	Quantity for 50µl of reaction mixture	Final Concentration
Sterile deionized water	variable	-
Pfu DNA Polymerase Buffer (Mg^{2+} plus)	5 µl	1X
dNTPs (10mM each）	1 µl	0.2 mM each
Primer I	variable	0.4 - 1 µM
Primer II	variable	0.4 - 1 µM
Pfu DNA Polymerase (5U/µl)	0.25 - 0.5 µl	1.25 - 2.5U/50 µl
Template DNA	variable	10 pg - 1 µg

Recommandations with Template DNA in a 50 µl 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. Mix contents of 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℃	3 minutes
25-35 cycles	94℃ 55-68℃ 72℃	30 seconds 30 seconds 1 minute
Final extension	72℃	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. Notes on cycling conditions :

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

Note :

- 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 incubation 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.

Store all components at –20°C