Differentiating Research

Product Details

Pfu DNA Polymerase, 5U/µL

(For High Fidelity)

Product Overview

Pfu DNA polymerase catalyzes DNA dependent polymerization from 5'→3', in presence of Mg2+ and also exhibits 3'→5' exonuclease activity for proof reading, which enables the polymerase to correct base insertion errors.

Catalog Details

R1201	500 U
R1202	2000 U

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Storage

- 20 ℃

PCR Protocol for templates ≤ 5KB

The samples must be prepared in sterile DNase Free micro-centrifuge tubes, with the following composition placed on **ice.**

Components	50µL reaction	Final Concentration
Template DNA	1 µL	10ng - 250ng
Forward Primer (10µM)	1µL	0.1 - 1µM
Reverse Primer (10µM)	1µL	0.1 - 1µM
dNTPs Mix (2.5mM Each)	4 µL	Upto 200 µM each
10X Pfu Reaction Buffer	5 µL	1X
DX/DT Pfu DNA Polymerase	0.5 µL	0.5 - 1.25 U
Nuclease Free Water	Upto 50 µL	

NOTES:

- Addition of all the above reagents should be done using ice boxes to prevent non-specific amplification.
- To collect all liquid at the bottom of the vial, reaction mix can be kept for a quick spin (10 seconds).
- While doing PCR without heat lid, it is recommended to overlay the sample with 1-2 drops of mineral oil.

Step	Temperature	Time	Cycle
Initial denaturation	98℃	30s	1
Denaturation	98℃	10s	25-35 Cycles
Annealing *	50 - 65 ℃	20s	
Extension**	72 ℃	1 minute/kb	
Final Extension	72 ℃	5-10 minutes	1
Hold, if required.	2- 8 ℃	variable	1

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Product Details

Pfu DNA Polymerase, 5U/µL

(For High Fidelity)

PCR Protocol for templates > 5KB

The samples must be prepared in sterile DNase Free micro-centrifuge tubes, with the following composition placed on ice

Components	50µL reaction	Final Concentration
Template DNA	1 µL	50ng - 250ng
Forward Primer (10µM)	1µL	0.1 - 1µM
Reverse Primer (10µM)	1µL	0.1 - 1µM
dNTPs Mix (2.5mM Each)	upto 6 µL	Upto 300 µM each
10X Pfu Reaction Buffer	5 µL	1X
DX/DT Pfu DNA Polymerase	0.5 µL	0.5 - 2.5µL
Nuclease Free Water	Upto 50 µL	

NOTES:

- Addition of all the above reagents should be done using **ice boxes** to prevent non-specific amplification.
- To collect all liquid at the bottom of the vial, reaction mix can be kept for a quick spin (10 seconds).
- While doing PCR without heat lid, it is recommended to overlay the sample with 1-2 drops of mineral oil.

Step	Temperature	Time	Cycle
Initial denaturation	98 ℃	30s	1
Denaturation	98 ℃	10s	25 - 35
Annealing *	50 - 65 ℃	20s	
Extension**	72 ℃	1 minute/kb	
Final Extension	72 ℃	10-20 minutes	1
Hold, if required.	2- 8 ℃	variable	1

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in ≥ 95% purity.
2. **Performance testing (1): 2.5**U of enzyme was used to amplify 10ng of DNA template (300bp, 1kb, 3kb and 5kb) in 30 PCR cycles resulted in a single band, confirmed by 1% agarose gel electrophoresis with EtBr.
3. **Performance testing (2)**: 2.5U of enzyme was used to amplify 50ng of DNA template (8kb) in 30 PCR cycles resulted in a single band, confirmed by 1% agarose gel electrophoresis with EtBr.
4. **Nuclease tests:** No contamination of endo or exonucleases were detected.

CRITICAL NOTE for Site Directed Mutagenesis

Please use 25 to 30 Cycles first to understand amplification results on Gel. Later keep 12- 14 cycles based on yield. Even if products are not visible on gel at low cycles, please go ahead for transformation. Lower the cycles better is fidelity.

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