Instructions for use

Pfu DNA polymerase, 5 U/µl

40 µl (200 units)

Recombinant Pfu DNA polymerase for high fidelity PCR amplifications, including 5´-> 3´ proofreading activity

1. Description

Recombinant version of the heat stable Pfu DNA polymerase from the extreme thermophilic archae bacterium *Pyrococcus furiosus* in storage buffer, plus additional 10x concentrated PCR-ProofRead reaction buffer.

For research use only. Not approved for use in clinical or *in vitro* diagnostics.

2. Applications

Pfu DNA polymerase is the optimal choice for all high-fidelity PCR assays, as being performed, for instance, prior to cloning or sequencing, in cycle sequencing or in site-directed mutagenesis reactions. In combination with the attached specially adjusted buffer, Pfu polymerase delivers sequence identical PCR amplificates of good yield with a wide range of PCR templates. Additionally, ***Pfu DNA polymerase*** is well suited for amplification of target sequences of high GC content, or with complex secondary structures.

Pfu DNA polymerase is able to amplify PCR products up to 3 kb with genomic DNA and is appropriate for use in the amplification of a broad variety of template DNAs. ***Pfu DNA polymerase*** included in the set replicates DNA 5´→ 3´ at 72 °C to 75 °C under presence of magnesium ions. Furthermore, ***Pfu DNA polymerase*** possesses a 5´→ 3´ (proof reading) exonuclease activity, rapidly substituting misincorporated bases during polymerization, and thus being responsible for the high sequence fidelity.

Pfu DNA polymerase generated DNA fragments are blunt-ended.

3. Set contents

Pfu DNA polymerase in storage buffer containing 50 % glycerol.
PCR ProofRead buffer (10x) with 20 mM $MgSO_4$.

The use of the colourless PCR reaction buffer is adequate for all general PCR applications.

Reagent	Amount	Lid colour
Pfu DNA polymerase, 5 U/µl	1 tube, 40 µl	orange
10x ProofRead buffer	1 tube, 1 ml	brown

4. Storage Buffer

50 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 0.5 % IGEPAL CA-630, 0.5 % Tween-20, 1 mM DTT, 0.05 % CHAPS, 50 % glycerol

5. Enzyme activity

5 units/µl enzyme solution

6. Unit definition

One unit of activity is defined as the amount of enzyme required to incorporate 10 nmoles of dNTP into an acid-insoluble DNA fraction in 30 minutes at 72 °C.

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7. Suggested pipetting scheme

At best prepare on ice:

Components	Apply for PCR reaction of 20 µl volume	Final concentration (recommended)
PCR ProofRead buffer (10x)	2 µl	1x
dNTP-Mix (2 mM)	2 µl	800 µM (200 µM each)
Forward primer (e.g. 5 pmol/µl)	variable (e.g. 1 µl)	0.1-0.5 µM
Reverse primer (e.g. 5 pmol/µl)	variable (e.g. 1 µl)	0.1-0.5 µM
Template DNA	variable	0.01-10 ng / reaction
Pfu DNA polymerase (5 U/µl)	variable (i.e. 0.2 µl)	0.5-1.5 U
Sterile dest. water	adjust to 20 µl final volume	

8. Basic amplification protocol

Step	Time	Temperature
Initial denaturation	2 minutes	92-95 °C
25-35 cycles		
Denaturation	2-10 seconds	92-95 °C
Annealing	2-10 seconds	55-68 °C
Extension	variable, depends on the length of product	72 °C

9. Notes

For maximum yield and specificity, annealing temperatures and annealing time as well as extension time and cycle numbers should be optimized for each template target and primer pair. Usually the optimal annealing temperature is 2-5 °C below the melting temperature of the primers. Recommended elongation time is 40 seconds per 1 kb of target. ***Pfu DNA polymerase*** has a slower processivity than standard Taq DNA polymerases. For a start, double the elongation time usually calculated for the use with Taq DNA polymerases. Elongation times may also depend on the complexity of template DNA.

10. Recommended $MgSO_4$ concentration

2 mM (final)

Generally, 2 mM $MgSO_4$ is suitable for most assays. However, in case the $MgSO_4$ concentration has to be adjusted, use a separate $MgSO_4$ solution (10 mM) in PCR quality and add in appropriate amounts according to the scheme below. We recommend doing PCR with a $MgSO_4$ gradient in order to find the optimal concentration.

Pipetting scheme for additional $MgSO_4$

Final $MgSO_4$ conc. in mM	2.5	3	3.5	4
Add 10 mM $MgSO_4$ solution in following amounts to 20 µl reaction volume	1 µl	2 µl	3 µl	4 µl

11. Storage conditions

Store the enzyme at -20°C. However, short term storage (few hours) of the enzyme may be done at ± 0°C (wet ice). The enzyme is also stable at room temperature for at least 3 days.
The buffer should be stored at -20°C, but may also be stored at +4 °C for several weeks.

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