Pfu DNA polymerase
, UltraPure (with dNTP)

Code No. DE131
Stored at -20℃

Kit Contents

Pfu DNA polymerase (2.5 units /µl, 100 units)	40 µl
10 × Reaction Buffer	1000 µl
dNTP Mixture	350 µl

Description

Pfu DNA polymerase is a high-fidelity thermostable DNA polymerase with 3'→5' exonuclease activity. The fidelity is more than ten-fold higher than that of *Taq* DNA polymerase. The highly purified *Pfu* DNA polymerase has no detectable contamination of host *E.coli* genome, guaranteeing less than 0.05 molecules of *E. coli* genome / unit (This figure reflects the detection limit of our present assay condition).

Source

E.coli cells carrying a cloned *Pfu* DNA polymerase.

Storage Buffer

50 mM Tris-HCl (pH 8.2), 1mM DTT, 0.1mM EDTA, 0.1% Tween® 20, 0.1% Nonidet P40® and 50 % glycerol.

10 x Reaction Buffer

200 mM Tris-HCl (pH8.8), 20 mM $MgSO_4$, 100 mM KCl, 100 mM $(NH_4)SO_4$, 1 % Triton X-100, 1 mg/ ml nuclease-free BSA

Contamination

The *Pfu* DNA polymerase (1 unit) has no detectable contamination of *E. coli* genomic DNA by high sensitive direct PCR with primers specific for *E. coli* 16S rRNA locus (less than 0.05 molecules of *E. coli* genome / unit). The *Pfu* DNA polymerase (2.5 unit) has no detectable ribonuclease and deoxyribonuclease contamination using RNaseAlert ® Kit and DNaseAlert™ Kit (Integrated DNA Technologies, Inc), respectively.

Fidelity

The fidelity of the *Pfu* DNA polymerase is determined as the mutation frequency according to the modified *rps*L assay described in (1). Gapped plasmid containing *rps*L reporter gene is filled by the *Pfu* DNA polymerase and then used to transform *E. coli* competent cell (*str*). Clones carrying mutated *rps*L appear on the streptomycin-containing plate. The mutation frequency of the *Pfu* DNA polymerase during a single round replication is about 0.03 %. This is more than ten- fold lower mutation frequency than that of *Taq* DNA polymerase.

Polymerase Activity

One unit of the *Pfu* DNA polymerase is defined as the amount of enzyme that catalyzes the incorporation of 200 pmols of dNTP into a hairpin oligomer (1) (as a template-primer) within 1 min at 65℃. The polymerase activity is assayed in a total volume of 100 µl reaction mixture containing 1 x Reaction Buffer, 200 µM dNTP and 0.4 µM hairpin oligomer. The amount of incorporated dNTP is determined from the initial rate of dsDNA synthesis by the *Pfu* DNA polymerase with SYBER® Green I according to the modified method described in (2).

PCR test

The *Pfu* DNA polymerase is tested for amplification of 0.5 kb, 1 kb, 2 kb, 4 kb, 6 kb and 8 kb fragments from λDNA as template.

PCR reaction mixture (50 µl)		(acceptable range)
10 x Reaction buffer	5 µl	
dNTP (2.5 mM each)	4 µl	(4 ul - 8 ul)
Forward primer (10 µM)	2 µl	(0.5 ul - 5 ul)
Reverse primer (10 µM)	2 µl	(0.5 ul - 5 ul)
Template DNA (for genomic)	100 ng	(50 ng - 1 µg)
(for plasmid)	or 1 ng	(50 pg - 50 ng)
Pfu DNA polymerase (2.5u/µl)	1 µl	(0.5 µl - 2 µl)
Water	up to 50 µl	

Thermal cycling condition

94℃	1 min	(94 - 98℃	30 sec - 3 min)	
94℃	30 sec	(94 - 98℃	15 sec - 1 min)	25-35 cycles
55℃	30 sec	(45 - 65℃	30 sec - 1 min)	
72℃	1 min / kb	(1 min - 2min / kb)		
72℃	10 min	(5 min - 15 min)		

Note

If keeping the high purity of the *Pfu* DNA polymerase is necessary, we recommend the use of a UV-irradiated cabinet without any air-flow to avoid air-borne contamination and the use of a positive-displacement micropipetter (e.g. Microman Gilson) instead of a conventional air-displacement micropipetter to avoid cross-contamination.

References

1. Nishioka M, et al. (2001) Long and accurate PCR with a mixture of KOD DNA polymerase and its exonuclease deficient mutant enzyme. J Biotechnol **88**: 141-9.
2. The sequence of the hairpin oligomer :5'-TACTCGGTGTACTCA CCGGTTCCGCAGATAGACATACTTATTAACTTATATTCAC TCTTACTTATACTCATCGATACTTTTGTATCGAT-3'
3. Bragin AG, et al. (2008) Determination of DNA polymerase and nuclease activities of DNA-dependent polymerases using fluorescence detection under real-time conditions. Biochemistry (Mosc) **73**:1007-17.

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