

# Life Technologies India Pvt. Ltd.

## Super Pfu DNA Polymerase (cloned)

## Pfu-100

## 5u/uL

## Storage : -20 deg C

**Product Description:** Super Pfu DNA polymerase is isolated from the hyperthermophilic marine archaeobacterium, *Pyrococcus furiosus*. The multifunctional thermostable enzyme possesses both 5'- to 3'- DNA polymerase and 3'- to 5'- exonuclease activity, which results in a 12-fold increase in fidelity of DNA synthesis over Pfu DNA polymerase. **Super Pfu DNA Polymerase has a temp. optimum between 72°C and 78°C remains > 95% active following a one hour incubation at 95°C.**

### 10 x Reaction Buffer:

200mM TrisHCl (pH 8.8)  
100 mM KCl  
100mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
20 mM Mg SO<sub>4</sub>  
1% Triton X-100  
1 mg / ml nuclease-free bovine serum albumin (BSA)

Unit Activity: One unit of activity is the amount of cloned Super Pfu DNA polymerase required to incorporate 10nM of [H]TTP into an acid-insoluble form in 30 minutes at 72°C.

**Storage Conditions: Super Pfu DNA polymerase is stored in 50mM Tris-HCL (pH8.2), 1mM dithiothreitol (DTT), 0.1mM EDTA, 0.1% Tween 20, 0.1% Nonidet P-40 and 50% (v/v) glycerol. Pfu DNA polymerase should be stored at -20°C on receipt.**

### Reaction Mixture Set Up--

- Gently vortex and briefly centrifuge all solutions after thawing.
- Add components, in the following order, into a thin-walled PCR tube. Keep all components on ice. The following control PCR reactions should be run in parallel to ensure that the Super Pfu DNA polymerase is working properly.

Reagent	Final Conc.	Quantity	Reagent	Positive Control	Negative Control
Water (PCR—Grade)	---	variable	Water (PCR—Grade)	32.8µl	33.8µl
10X Pfu reaction buffer	1X	5µl	10X Pfu reaction buffer	5µl	5µl
2.5mM dNTP mixture	200µM of each	4µl	2.5mM dNTP mixture	4µl	4µl
Primer I, forward	0.1-1µM	variable	Primer I (10µM), forward	1µl	1µl
Primer II, reverse	0.1-1µM	variable	Primer II (10µM), reverse	1µl	1µl
Pfu DNA polymerase	1-1.5u/50µl	Variable	Pfu DNA polymerase	0.2µl	0.2µl
Template DNA	See note 1	Variable	Control DNA Template	----	----
<b>Total Volume</b>	----	50µl	<b>Total Volume</b>	50µl	50µl

- Gently vortex the sample and briefly centrifuge to collect all drops from walls of the tube.
- Overlay the sample with one-half of the total reaction volume of mineral oil or add an appropriate amount of wax. This step may be omitted if the thermo cycler is equipped with a heated lid.
- Place samples in a thermo cycler and start **PCR**.

### Note for the Components of the Reaction Mixture:

- Template DNA:** Usually the amount of template DNA is in the range of 0.01- 1ng plasmid or phage DNA & 0.1-1µg for genomic DNA, for a total reaction mixture of 50µl.
- Primers:** The PCR primers are usually 15-30 nucleotides in length, longer primers provide higher specificity. The GC content of primer should be 40-60%. The primer should not be self-complementary or complementary to any other primer in the reaction mixture, and the melting temperature of flanking primers should not differ by more than 5°C. If the primer is shorter than 25 nucleotides, the approx. melting temperature, **T<sub>m</sub>** is calculated using the formula as: **T<sub>m</sub>=4(G+C) + 2(A+T)**.
- dNTPs:** The final concentration of each dNTP in the reaction mixture is usually 200µM.
- Super Pfu DNA polymerase:** Usually 1-15u of Super Pfu DNA polymerase are used in the 50µl of reaction mix. Higher Super Pfu DNA polymerase concentrations may cause synthesis of nonspecific products. However, if inhibitors are present in the reaction mix (e.g., if the template DNA used is not highly purified), higher amounts of Pfu DNA polymerase (2-3u) may be necessary to obtain a better yield of amplification products.
- Usually the extending step is performed at 70-75°C. Super Pfu DNA Polymerase exhibits lower extension rate than *Taq* DNA Polymerase, so 2min extension time is recommended for every 1 kb to be amplified.
- Cycling conditions:** Usually denaturation for 0.5-2min at 94-95°C is sufficient; the optimal annealing temperature is 5°C lower than the melting temperature of primer-template DNA duplex; Usually the extending step is performed at 70-75°C. Recommended extending time is 1 min for the synthesis of PCR fragments up to 2kb. When larger DNA fragments are amplified, the extending time is usually increased by 1 min for each 1kb.
- Number of cycles:** 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. For less than 10 copies of template DNA, 40 cycles should be performed. If the initial quantity of template DNA is higher, 25-35 cycles are usually sufficient.
- Final extending step:** After the last cycle, the samples are usually incubated at 72°C for 5-15min.

### Marketed by:

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