Product information

Cloned Pfu DNA Polymerase

Catalog #: B0093
Quantity: 500U
Concentration: 5 U/ul
Specific Activity: 15,000U/mg
Storage: -20°C
Certified by: Jennifer Wang (This certificate is a declaration of analysis at the time of manufacture).
Expiration: Approximately 12 months after the product is received.
Package:

Cloned Pfu DNA Polymerase	100U	500U
10 x Rxn. Buffer	1.8ml	2x1.8ml

Product Description:

Cloned Pfu DNA polymerase is isolated from the hyperthermophilic marine archaebacterium. 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. Cloned Pfu DNA Polymerase has a temperature optimum between 72°C and 78°C remains > 95% active following one hour incubation at 95°C.

10 x Reaction Buffer:

200 mM TrisHCl (pH 8.8)
100 mM KCl
100 mM (NH4)2 SO4
20 mM MgSO4
1% Triton X-100
1 mg / ml nuclease-free bovine serum albumin (BSA)

Unit Activity:

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

Contaminants:

Cloned Pfu DNA polymerase is tested extensively for the absence of detectable nonspecific nuclease activity and DNA contamination.

Shipping Conditions:

Cloned Pfu DNA polymerase is shipped in gel package. But remains full activity at room temperature.

Storage Conditions:

Cloned 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. Cloned Pfu DNA polymerase should be stored at -20°C on receipt.

Limited Product Warranty:

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BIO BASIC INC. We shall have no liability for any direct, indirect, consequential or incidental damages arising out of the use, the results of use, or the inability to use this product.

⚠ ***Disclaimer:*** *This product has not been licensed for use in the polymerase chain reaction (PCR) process for amplifying nucleic acids. (US patent numbers 4683195 and 4683202 issued to Cetus)*

Reaction Mixture Set Up:

1. Gently vortex and briefly centrifuge all solutions after thawing.

2. 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 Pfu DNA polymerase is working properly.

Reagent	Final Concentration	Quantity		Reagent	Positive Control	Negative Control
Water (PCR--Grade)	---	variable		Water (PCR--Grade)	32.8ul	33.8ul
10x Pfu reaction buffer	1x	5ul		10x Pfu reaction buffer	5ul	5ul
2.5mM dNTP mixture	200uM of each	4ul		2.5mM dNTP mixture	4ul	4ul
Primer I, forward	0.1-1uM	variable		Primer I (10uM), forward	1ul	1ul
Primer II, reverse	0.1-1uM	variable		Primer II (10uM), reverse	1ul	1ul
Pfu DNA polymerase	1-1.5U/50ul	variable		Pfu DNA polymerase	0.2ul	0.2ul
Template DNA	See note 1	variable		Control DNA Template	1ul	---
Total Volume	---	50ul		Total Volume	50ul	50ul

3. Gently vortex the sample and briefly centrifuge to collect all drops from walls of the tube.

4. 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.

5. Place samples in a thermo cycler and start PCR.

Notes for the Components of the Reaction Mixture:

1. Template DNA: Usually the amount of template DNA is in the range of 0.01-1ng plasmid or phage DNA and 0.1-1ug for genomic DNA, for a total reaction mixture of 50ul.

2. 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 (Tm) is calculated using the formula as: Tm = 4(G+C) + 2(A+T).

3. dNTPs: The final concentration of each dNTP in the reaction mixture is usually 200uM.

5. Pfu DNA polymerase: Usually 1-1.5U of Pfu DNA polymerase is used in the 50ul of reaction mix. Higher 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.

6. Usually the extending step is performed at 70-75°C. Pfu DNA Polymerase exhibits lower extension rate than Taq DNA Polymerase, so 2min extension time is recommended for every 1kb to be amplified.

7. 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 1min for the synthesis of PCR fragments up to 2kb. When larger DNA fragments are amplified, the extending time is usually increased by 1min for each 1kb.

7. 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.

8. Final extending step: After the last cycle, the samples are usually incubated at 72°C for 5-15min.

