GPfu DNA Polymerase (Recombinant)

 # G7122 100Units (1U/µl)

Store at -20°C

Spin tubes briefly before use

Catalogue Number	Pack Size	☑
G7122	100 Units	✓
G7122A	500 Units	

Package information

Component	G7122	G7122A
GPfu DNA Polymerase Recombinant (1 unit/λ)	100 Unit	5 X 100 Unit
10X GPfu Buffer	(1 X 625 µl)	(5 X 625 µl)
45mM $MgCl_2$	(1 X 625 µl)	(5 X 625 µl)
Control DNA tempalate	10 µl	10 µl
Control Primer mix	10 µl	10 µl

Introduction

GPfu DNA Polymerase is a highly purified thermostable DNA polymerase from the hyperthermophilic archaeum Pyrococcus furiosus. This GPfu DNA Polymerase (recombinant) is the pol gene from Pyrococcus furiosus – cloned and expressed in bacteria. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction. Prime Pfu DNA Polymerase exhibits 3'→5' exonuclease (proofreading) activity that enables the polymerase to correct nucleotide incorporation errors. It has no 5'→3' exonuclease activity. Amplify upto 4kb.

Applications

- High fidelity PCR.
- Generation of PCR products for cloning and expression.
- RT-PCR for cDNA cloning and expression.
- Generation of PCR product for blunt-end cloning.
- Site-directed mutagenesis.

Storage Buffer

The enzyme is supplied in: 20mM Tris-HCl (pH 8.2), 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.1%(v/v) Nonidet P40, 0.1%(v/v) Tween 20 and 50%(v/v) glycerol.

Protocol

Prepare a PCR master mix by mixing water, buffer, dNTPs, primers and template DNA. GPfu DNA polymerase should be the last component to add. Prepare sufficient master mix for the number of reactions plus one extra to avoid pipetting error.

1. Gently vortex and briefly centrifuge all solutions after thawing except GPfu Polymerase.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 µl reaction:

Component	Sample	Control
Water, Nuclease free	Up to 50µl	36µl
10X G*Pfu* Buffer	5µl	5µl
45mM $MgCl_2$	5µl	5µl
10mM dNTP mix	1µl	1µl
Forward Primer (10µM)	1µl	1µl from the primer mix
Reverse Primer (10µM)	1µl	
Template DNA	5pg-800ng (varies with complexity)	1µl
G*Pfu* DNA Polymerase	1-2µl	1µl (1U)
Total Volume	50µl	50µl

3. Gently vortex the samples and spin down.
4. If using a thermal cycler that does not use a heated lid, overlay the reaction mixture with 25 µl of mineral oil.
5. Perform PCR using the following thermal cycling conditions:

Table 2. Cycling Instructions

Cycle step	2-step Protocol		Cycles
	Temp.	Time	
Initial Denaturation	94°C	5min	1
Denaturation	94°C	15-30sec	25-35
Annealing	Tm	15-60sec	
Extension	68°C-72°C	60sec/1kb	
Final extension	68°C	5-15 min	1

Cycling Parameters

Step	Temperature (°C)	Time	No. of Cycles
Initial denaturation	94	1-3 min (3 min for Control PCR)	1
Denaturation	94	30 sec	25-35
Annealing	T_m-5 (57°C for control PCR)	30 sec	
Extension	68	2 min/kb	
Final extension	68	5-15 min	1

*** A 2kb band will be obtained for control PCR.**

Notice

1. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 94°C is sufficient. For GC-rich templates this step should be prolonged up to 10 min.

2. A DNA denaturation time of 30 seconds per cycle at 94°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

3. Annealing for 15 seconds is normally sufficient. If non-specific or no PCR products appear, the annealing temperature should be optimized.

4. The optimal extension temperature for GPfu DNA Polymerase is 68-72°C. The recommended extension step is 2 min/kb at 72°C for PCR products up to 4 kb. For larger products, the extension temperature should be 68^{O}C.

5. The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield. In most cases, 25-35 cycles are sufficient.

6. After the last cycle, it is recommended to incubate the PCR mixture at 72°C for PCR products up to 2 kb and for larger products, the temperature should be 68^{O}C for additional 5-15 min to fill-in any possible incomplete reaction products.