Pro Pfu DNA Polymerase (Pfu Turbo)

 # G7203 **100U (1U/µl)**

Storage: -20^{0}C

Spin tubes briefly before use

Description

Pro *Pfu* DNA polymerase is an enhanced version of *Pfu* DNA polymerase for robust, high-fidelity PCR. Pro *Pfu* DNA polymerase is a mixture of recombinant *Pfu* DNA polymerase and the exclusive thermostable ArchaeMaxx polymerase-enhancing factor that enhances PCR product yields and increases target length capability without altering DNA replication fidelity. The enhanced performance of Pro *Pfu* DNA polymerase allows using fewer PCR cycles and lower DNA template concentrations, as compared to Pfu DNA polymerase. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction. Pro 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.

Kit contents:

Materials	Amount
Pro Pfu DNA Polymerase (Pfu Turbo)	100U
10X Pro Pfu Buffer	625µl
25mM $MgCl_2$	625µl
Control forward and reverse primers mix for 8kb PCR	10 µl
Control template	10 µl

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: 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 100 mM KCl, 0.1% (v/v) IGEPAL, 0.1% (v/v) Tween 20 and 50% (v/v) glycerol.

10X Pro Pfu Buffer

200 mM Tris-HCl (pH 8.8 at 25°C), 100 mM $(NH4)_2SO_4$, 0.1% Tween 20

Protocol

Prepare a PCR master mix by mixing water, buffer, dNTPs, primers and template DNA. Pro Pfu 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 Pro Pfu 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	Variable	37.5 µl
10X Pro Pfu Buffer	5 µl	5 µl
25mM $MgCl_2$	5µl	5µl
dNTP mix, 10 mM each	1 µl	1 µl
Forward Primer	0.1-1.0 µl	2 µl from the primer mix
Reverse Primer	0.1-1.0 µl	
Template DNA	50 pg-1 µg	4 µl (1 ng)
Pro Pfu DNA Polymerase	1.25-2 u	1 µl
Total Volume	50 µl	50 µl

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:

CYCLING PARAMETERS

Step	Temperature (°C)	Time	No. of Cycles
Initial Denaturation	94	1-3 min (3 min for Control PCR)	1
Denaturation	94	15 sec	25-30
Annealing	Tm-5 (590C for control PCR)	15 sec	
Extension	68	2 min/kb	
Final extension	68	10-20 min	1

Notes:

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 15 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 *Pro Pfu DNA* Polymerase is 68°C. The recommended extension step is 1.5 -2 min/kb at 68°C for PCR products up to 16 kb and above depending upon the DNA template and amplicon size.

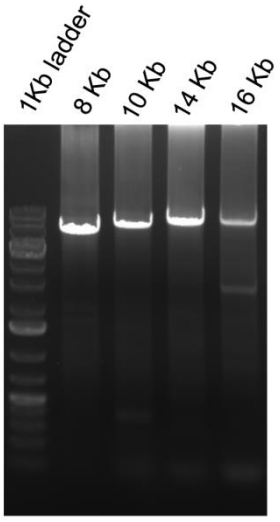
5. The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield. In most cases, 25-35 cycles are sufficient.

6. For larger DNA amplicon (> 8KB) enzyme conc. be used as 0.75µl for 50µl reaction volume.

7. 1.5-2mins/kb elongation time for long amplicon may improve the amplification quality.

Storage and handling:

1. Store at -20ºC upon arrival.
2. Minimize number of freeze thaw cycle by storing it in working aliquots.



8 - 16 kb fragments are amplified with Pro *pfu* DNA polymerase. 1/5 volume of the pcr amplified product analysed in 1% agarose gel.

Troubleshooting

No product at all or low yield	
• **Optimize annealing temperature.** • **Repeat and make sure that there are no pipetting errors.** • **Make sure the cycling protocol was performed as recommended.** • **Use fresh high-quality dNTPs.** • **Do not use dNTP mix or primers that contain dUTP or dITP.** • **Titrate template amount. Template DNA may be damaged. Use freshly prepared template.**	• Increase extension time. • Increase the number of cycles. • Titrate DMSO (2−8 %) in the reaction. • Denaturation temperature may be too low. Optimal denaturation temperature for most templates is 94-98°C. • Optimize denaturation time. • Check the purity and concentration of the primers. • Check primer design.
Non-specific products - High molecular weight smears	
• **Make sure the extension time used was not too long.** • **Reduce the total number of cycles.** • **Increase annealing temperature or try 2-step PCR protocol.**	• Titrate template amount. • Optimize denaturation temperature. • Decrease primer concentration.
Non-specific products - Low molecular weight discrete bands	
• **Increase annealing temperature** • **Make sure the extension time used was not too long.**	• Titrate template amount. • Decrease primer concentration. • Design new primers.