GENAXXON bioscience

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Pfu DNA Polymerase

Deoxynucleoside-triphosphate: DNA deoxynucleotidyl-transferase (DNA-directed); EC 2.7.7.7

	Cat#	M3004.0250	M3004.0500	M3004.1250	Colour code of cap
Component					
***Pfu* DNA Polymerase (proof-reading polymerase)**		250 units	2x 250 units	5x 250 units	**colourless**
5X PCR Buffer with MgSO4		1mL	1mL	5x 1mL	**purple**

Description

The Genaxxon bioscience *Pfu* DNA Polymerase is a thermostable enzyme possessing 5'-3' DNA polymerase and 3'-5' proof reading exonuclease activities. It is isolated from the hyperthermophilic marine archae *Pyroccocus furiosus* (*Pfu*). The enzyme provides extremely high fidelity. Whereas the enzyme is not able to amplify long fragments as efficiently as the Genaxxon *Taq* polymerase because of its very high exonuclease activity. To overcome this restriction, we recommend to use the Genaxxon ReproFast DNA Polymerase, which will provide a more robust synthesis of longer amplification products (Barnes et al. (1994) Proc. Natl. Acad. Sci., USA 91, 2216-2220). Use of the Genaxxon *Pfu* DNA Polymerase in amplification results in blunt-ended products, which is not recommended for cloning into T/A vectors.

Concentration: 2.5 units/µL

Unit definition: One unit is defined as the amount of enzyme that incorporates 10nmoles of dNTPs into acid-insoluble form in 30 min at 72°C under the assay conditions (25mM TAPS (tris-(hydroxymethyl)methyl-amino-propane-sulphonic acid, sodium salt) pH9.3 (at 25°C), 50mM KCl, 2mM MgCl2, 1mM ß-mercaptoethanol) and activated calf thymus DNA as substrate.

Storage: -20°C

Storage buffer: 50mM Tris-HCl, pH8.2 (25°C), 0.1mM EDTA, 1mM DTT, 0.1% Nonidet® -P40, 0.1% Tween® 20, 50% Glycerol.

10X Reaction buffer: 60mM $(NH_4)_2SO_4$, 200mM Tris-HCl pH8.8 (at 25°C), 100mM KCl, 20mM MgSO4, 1% Triton X100, 1% BSA

The 10X reaction buffer is delivered free of charge.

Product Specifications

Concentration:	2.5 units/µL
Substrate analogs:	dNTP, ddNTP, fluorescent dNTP/ddNTP
Extension rate:	0.5 - 1 kb/min. at 72°C
Half-life:	20min. at 95°C, 60min. at 94°C
5'-3' exonuclease activity:	No
Extra addition of A:	No
3'-5' exonuclease activity:	Yes
Nuclease contamination:	No
Protease contamination:	No
RNase contamination:	No
Self-priming activity:	No
Storage and dilution buffer:	20mM Tris-HCl (pH 8.3), 100mM KCl, 0.1mM EDTA, 1mM DTT, 50% glycerol, 0.5% Nonidet P40 and 0.5% Tween 20

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Quality Control

Amplification efficiency:	Amplification efficiency is tested in parallel amplification reactions and additionally against competitors' products.
PCR reproducibility:	PCR reproducibility is tested in parallel amplification reaction.
Exonuclease activity:	Linearized DNA is incubated with *Pfu* DNA Polymerase and *Pfu* buffer.
Endonuclease activity:	Plasmid DNA is incubated with *Pfu* DNA Polymerase and *Pfu* buffer.
RNase activity:	RNA is incubated with *Pfu* DNA Polymerase and Pfu buffer.
Protease activity:	*Pfu* DNA Polymerase is incubated in storage buffer.
Self-priming activity:	PCR is performed under standard conditions, without primers, using *Pfu* DNA Polymerase and human genomic DNA.
Application:	High fidelity amplification Primer extension reactions PCR cloning and blunt-end amplification product generation
Usage:	**Cycle times especially extension times should be extended, compared to *Taq* DNA polymerase.** **Note:** Recommended elongation time is 1 minute per 250bp of target!

Supplied buffers/solutions: *- 5X PCR buffer with Mg2SO4*

Stability

Genaxxon bioscience *Pfu* DNA Polymerase is shipped on wet ice but retain full activity at +15°C to +30°C for at least 2 weeks.

Pfu DNA Polymerase including buffers and reagents, should be stored immediately upon receipt at -20°C.
When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance. The Genaxxon bioscience *Pfu* DNA Polymerase can also be stored at +2°C to +8°C up to 3 months.

Product Use Limitations

Pfu DNA Polymerase is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Safety information

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com)

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PCR Protocol Part

Protocol using *Pfu* DNA Polymerase

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

Important notes before getting started
Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Things to do before starting

- If required, prepare a dNTP-mix containing 10mM of each dNTP (or ready-to-use product M3016 from Genaxxon bioscience). Store this mix in aliquots at -20°C.

Procedure

1. **Thaw 10X buffer, dNTPs or dNTP-mix and primer solutions** at RT or on ice.
   Keep the solutions on ice after complete thawing. Mix well before use to avoid localized differences in salt concentration.
2. **Prepare a master mix according to Table 1.**
   The master mix typically contains all of the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included in every experiment.
   Keep the master mix on ice.

Table 1: PCR reaction components (**50µL PCR reaction**)

Components	Quantities
Pfu DNA Polymerase	0.3 – 0.8µL (0.5 – 2.0 units)
10X PCR buffer	5µL
Nucleotides	1µL (10mM) each dATP, dCTP, dGTP, dTTP or 1µL of 10mM ready-to-use dNTP mix.
primer 1:	0.5µL (0.25 – 2.5µL) 0.1µM solution (0.05 - 0.5µM (5 – 25 pmol absolute))
primer 2:	0.5µL (0.25 – 2.5µL) 0.1µM solution (0.05 - 0.5µM (5 – 25 pmol absolute))
Template DNA	<10ng plasmid DNA or <500ng genomic DNA
sterile, bidestilled water	up to 50µL

3. **Mix the master mix thoroughly**, and dispense appropriate volumes into PCR tubes.
   Mix gently, for example, by pipetting the master mix up and down. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.
4. **Add template DNA** to the individual tubes containing the master mix.
   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of final PCR volume.
5. **Program the thermal cycler** according to the manufacturer's instructions.
   A typical PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.
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Table 2: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	3 min.	94°C	
3-step cycling			
Denaturation:	0.5 - 1 min.	94°C	
Annealing:	0.5 - 1 min.	50 - 68°C	Approximately 5°C* below lower Tm of primers.
Extension:	0.5 - 1 min.	72°C	For PCR products longer than 1kb, use an extension time of approximately 1min./kb DNA.
Number of Cycles	25 - 35		
Final extension	10 min.	72°C	

Note: After amplification, samples can be stored at 4°C overnight, or -20°C for long term storage.

1. **For simplified hot start**, proceed as described in step 8. Otherwise, place the PCR tube in the thermal cycler and start program.
2. **Simplified hot start:** Start PCR program. Once the thermal cycler has reached 94°C, place PCR tubes in the thermal cycler. In many cases, this simplified hot start improves specificity of PCR.
   For highly specific and convenient hot start PCR please use the Genaxxon bioscience HotStart DNA Polymerase (M3006), SuperHot Taq DNA Polymerase (M3307) or the HotStart Master mix (M3007).

Table 3: Recommendations for Standard PCR-Primers

Length:	18-30 nucleotides
GC-Content:	40-60%
Tm:	Design primer pairs with similar Tm values.
	Optimal annealing temperature may be above OR below the estimated Tm. As a starting point, use an annealing temperature of 3°C to 5°C below Tm of the primer with the lower Tm-Value.
Sequence:	Avoid complementarities of two or more bases at the 3' ends of primer pairs.
	Avoid runs of 3 or more Gs or Cs at the 3' end.
	Avoid a 3'-end T.
	Avoid complementary sequences within primer and between primer pairs.

Table 4: Migration Chart of some Gel Tracking Dyes

Dye in agarose gel	0.5%-1.5%	2.0%-3.0%	CAS-number	Cat-No. Genaxxon
Xylene cyanol	10000bp - 4000bp	750bp - 200bp	2650-17-1	M3312
Cresol Red	2000bp - 1000bp	200bp - 125bp	62625-29-0	M3371
Bromophenol blue	500bp - 400bp	150bp - 50bp	115-39-9	M3092
Orange G	<100bp	<20bp	1936-15-8	M3180
Tartrazine	<20bp	<20bp	1934-21-0	

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