

# Genaxxon BioScience

## *Pwo*-DNA Polymerase

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

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Product	Cat#	Package size
<i>Pwo</i> -DNA Polymerase (proof-reading polymerase)	M3002.0100	100 units
<i>Pwo</i> -DNA Polymerase (proof-reading polymerase)	M3002.0500	2 x 250 units
<i>Pwo</i> -DNA Polymerase (proof-reading polymerase)	M3002.1250	5 x 250 units

### Description

The Genaxxon BioScience *Pwo* 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 *Pyrococcus woesei* (*Pwo*). 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 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 *Pwo*-DNA polymerase in amplification results in blunt-ended products, ideal for cloning into blunt-end vectors like the Genaxxon pMBL cloning vector.

**Usage:** As Taq-DNA polymerase. Cycle-times especially extension times should be extended.

**Application:** High fidelity amplification.  
 Primer extension reactions  
 PCR cloning and blunt-end amplification product generation

**Concentration:** 2.5 units/ $\mu$ L

**Unit definition:** One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 min at 72°C under the assay conditions (25 mM TAPS (tris-(hydroxymethyl)methyl-amino-propane-sulphonic acid, sodium salt) pH 9.3 (at 25°C), 50 mM KCl, 2 mM MgCl<sub>2</sub>, 1 mM  $\beta$ -mercaptoethanol) and activated calf thymus DNA as substrate.

**Storage:** -20°C

**Storage buffer:** 20 mM Tris-HCl (pH7.9; 25°C), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, 50% Glycerin.

**10X Reaction buffer: (complete)** 100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 200 mM Tris-HCl pH 8.8 (at 25°C), 100 mM KCl, 20 mM MgSO<sub>4</sub>, 1% Triton X100, 1% BSA (nuclease free).

The 10X reaction buffer is delivered free of charge.

**Quality control:** Activity, SDS-PAGE purity, absence of endonucleases/nickases

**Note:** Recommended elongation time is 1 minute per 250 bp of target!

## Suggested PCR Protocol

Pipette the following into a PCR tube, mix and make up to a final volume of 50µL. We recommend dispensing all reagents on ice, adding the enzyme last. It is important to vortex all buffers and MgCl<sub>2</sub> solutions before use to remove any gradients that may result from repeated freeze/thaw steps. If you do have already your own PCR-Protocol established, please use your existing pipetting scheme and Thermocycler protocol.

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Components	Quantities
Template DNA	1 ng - 10 ng plasmid DNA or 5 ng - 500 ng genomic DNA
Nucleotides	1µL (10 mM) each dNTP
10X amplification buffer	5µL
primer 1:	4-7µL of 3 µM solution (10 - 20 pmol absolute)
primer 2:	4-7µL of 3 µM solution (10 - 20 pmol absolute)
sterile, bidistilled water	up to 50µL
<i>Pwo</i> -Polymerase	0.3 - 0.8µL (0.5 - 2.0 units)

The following cycling protocol has to be seen as suggestion. Cycle numbers and cycle times have to be adjusted.

Cycling Profile *	
94°C for 2-4 minutes	step 1(denaturing of DNA)
	(cycling part)
94°C for 30-60 sec.	1 - 30 cycle(s)
annealing temp. for 30-60 sec.	1 - 30 cycle(s)
extension (72°C) for 60-240 sec. **	1 - 30 cycle(s)
	final step (filling up step)
94°C for 30 sec.	
72°C for 5-10 minutes	
cool down to 4°C	

\* cycling times are proposals and have to be adjusted to the special needs.

\*\* the longer the template to be amplified, the longer the amplification time

After running the amplification reaction, 5 µL of each sample should be analyzed in a 1% agarose gel containing 0.5µg/mL of ethidium bromide.

**Note:** For every template/primer pair the optimal reaction conditions have to be evaluated empirically, changing the primer/template ratio, the ionic strength (with MgSO<sub>4</sub>) and the cycle parameters (time and temperatures).

## Trouble shooting:

### No PCR® Product

Check if annealing temperature has been calculated correctly. The optimal annealing temperature of primers lies about 5°C below the T<sub>M</sub> of the oligo.

TM calculation: For oligos up to 15 bases:  $TM = 4x(G+C) + 2x(A+T)$   
 For larger oligos:  $TM = 81,5+16,6([\log_{10}J^*]) + 0,4(\%G+C) - (600/n) - 0,63(\%FA)$

A = Adenosine, C = Cytosine, G = Guanine, T = Thymidine, FA = Formamide, J\* = concentration of monovalent cations, n = number of bases; T<sub>M</sub> = calculated melting temperature,

**Remark:** If primers with mismatches are used the *Pwo* Polymerase must be added immediately before the start of the PCR®. Do not let the reaction mix stand at room temperature since primers will be degraded by the *Pwo* Polymerase.

### Bands smear over the Gel

- Increase annealing temperature
- Add up to 5% DMSO to the Reaction mixture
- Reduce the amount of template DNA
- Check if the primers bind more than once on the template DNA. Perform control reactions with only one of the primers.

### Low Yields

- Increase annealing temperature

The PCR process is licensed and covered under US-Patent owned by Hoffmann-La Roche, Roche Int. USA

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