

Genaxxon BioScience

LongMax PCR Kit (up to 20 kb)

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Product	Cat#	Package size
LongMax PCR Kit (up to 20kb)	M3000.0100	100 units
LongMax PCR Kit (up to 20kb)	M3000.0250	250 units
LongMax PCR Kit (up to 20kb)	M3000.2500	5 x 500 units

Product description

The Genaxxon LongMax PCR Kit is designed for high fidelity PCR. A mixture from the Genaxxon Taq-Polymerase with the Genaxxon Pfu-Polymerase together with an adopted buffer system and specially tested dNTPs guarantees amplification of DNA up to 15 kb (genomic DNA) or 20 kb (lambda DNA). The use of our LongMax PCR Kit results in DNA fragments that contain A-overhangs (up to 70%) thus allowing T/A cloning with the Genaxxon T/A cloning system (M3164).

The LongMax PCR Polymerase mixture catalyses the polymerisation of DNA in 5' -> 3' direction in the presence of magnesium ions and possesses a 5' -> 3' exonuclease activity. It also shows 3' -> 5' exonuclease activity (proof-reading).

Supplied material

- LongMax Polymerase mixture (5 units/μl)
- dNTP-solution (2 mM per dNTP)
- 10 x PCR buffer with MgCl₂
- Magnesium stock solution (25 mM)
- Positive DNA control (25 ng/ml)
- Primer for positive control (15 pmole/μl)

Unit definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP's into acid-insoluble fraction in 30 minutes at 74°C under the standard assay conditions: 25 mM TAPS (tris-(hydroxymethyl)-methyl-amino-propansulfonic acid, sodium salt) pH 9,3 (at 25°C), 50 mM KCl, 2 mM 50 mM MgCl₂, 1 mM β-mercapto-ethanol, 200 μM each dATP, dGTP, dTTP, 100 μM dCTP (a mix of cold and P32-labelled), 12,5 μg activated salmon sperm DNA, in a final volume of 50 μl.

Storage and dilution buffer

20 mM Tris-HCl (pH 8,0), 100 mM KCL, 0,1 mM EDTA, 1 mM DTT, 50% glycerol, 0,5% Nonidet P40 and 0,5% Tween 20.

Stability

The enzyme is stable for more than 12 months if stored at -20°C. The enzyme is also stable for some days at temperatures above 20°C.

Associated activities

Endonuclease and exonuclease activities were not detectable after 4 hours incubation of 1 μg native lambda DNA and 0.22 μg of EcoR I-digested lambda DNA at 72°C in the presence of 15 - 20 units of Genaxxon Taq-DNA Polymerase.

Properties and application

The Genaxxon LongMax PCR Kit is recommended for amplification of large fragments up to 20 kb. The enzyme mixture produces A-overhangs at the end of the amplified DNA, thus allowing T/A cloning.

Standard DNA amplification assay

Pipette the following into a PCR tube, mix and make up to a final volume of 50 µl. We recommend dispensing all reagents on ice, adding the enzyme last. It is important to vortex all buffers and MgCl₂ 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.

Pipetting scheme

Components	Quantities
Template DNA	x µl DNA (50 ng plasmid DNA (or 300 ng – 500 ng genomic DNA))
2 mM dNTPs	5.0 µl
10X amplification buffer	5.0 µl
25 mM MgCl ₂	5.0 µl
primer 1:	1 µl of 15 µM solution (15 pmole/µl)
primer 2:	1 µl of 15 µM solution (15 pmole/µl)
LongMax Polymerase mixture	1 µl (5 units)
sterile, RNase and DNase free water	up to 50 µl

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Drops should be collected by centrifugation and 50 µl of mineral oil (m3024.0810) should be layered upon the reaction mixture.

PCR programme

Cycling Profile – DNA Template	
94°C for 5 minutes	step 1
94°C for 35 sec.	1 - 35 cycle(s)
annealing temp. for 35 sec.	1 - 35 cycle(s)
extension at 68°C, 1 min. per 1 kb to be amplified. **	1 - 35 cycle(s)
94°C for 35 sec.	
68°C for 7 minutes	last step
cool down to 4°C	

Cycling Profile – Control DNA ***	
94°C for 5 minutes	step 1
94°C for 35 sec.	1 - 35 cycle(s)
60°C for 30 sec.	1 - 35 cycle(s)
extension at 68°C, 7 min	1 - 35 cycle(s)
94°C for 35 sec.	
68°C for 7 minutes	last step
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.

*** The amplified control DNA is expected to show a size of about 14 kb on a 0.7% agarose gel.

Note: For every template/primer pair the optimal reaction conditions have to be evaluated empirically, changing the primer/template ratio, the ionic strength (with MgCl₂) 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_m 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; TM = calculated melting temperature,

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

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PCR protocol for control DNA

Pipette the following into a PCR tube, mix and make up to a final volume of 50 µl. We recommend dispensing all reagents on ice, adding the enzyme last. It is important to vortex all buffers and MgCl₂ 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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Pipetting scheme

Components	Quantities
Control DNA	1 µl DNA (50 ng/µl plasmid DNA as delivered) (or 300 ng – 500 ng genomic DNA)
2 mM dNTPs	5.0 µl
10X amplification buffer	5.0 µl
25 mM MgCl ₂	5.0 µl
Control primer mix:	2 µl of 15 µM solution (15 pmol/ml each)
LongMax Polymerase mixture	1 µl (5 units)
sterile, RNase and DNase free water	up to 50 µl

Drops should be collected by centrifugation and 50 µl of mineral oil (m3024.0810) should be layered upon the reaction mixture.

PCR programme

Cycling Profile – Control DNA ***	
94°C for 5 minutes	step 1
94°C for 35 sec.	1 - 35 cycle(s)
60°C for 30 sec.	1 - 35 cycle(s)
extension at 68°C, 7 min	1 - 35 cycle(s)
94°C for 35 sec.	
68°C for 7 minutes	
cool down to 4°C	last step

*** The amplified control DNA is expected to show a size of about 14 kb on 0.7% agarose gel.

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A = Adenosine, C = Cytosine, G = Guanine, T = Thymidine, FA = Formamide, J* = concentration of monovalent cations, n = number of bases; TM = calculated melting temperature,

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

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