

Genaxxon BioScience

DF-Taq DNA-Polymerase (DNA-free Taq)

Deoxynucleosidetriphosphate: DNA nucleotidyltransferase from *Thermus aquaticus*

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Product	Cat#	Package size
DF-Taq DNA Polymerase with buffer and MgCl ₂	M3185.0250	250 units
DF-Taq DNA Polymerase with buffer and MgCl ₂	M3185.0500	2 x 250 units
DF-Taq DNA Polymerase with buffer and MgCl ₂	M3185.1000	4 x 250 units
DF-Taq DNA Polymerase with buffer and MgCl ₂	M3185.2500	10 x 250 units

Product description

The Genaxxon BioScience DF-Taq DNA Polymerase is a highly processive 5' - 3' DNA Polymerase, lacking 3' - 5' exonuclease activity. The high processivity and fidelity of Genaxxon BioScience Taq Polymerase allows amplification of DNA fragment of up to 10 kb. Genaxxon BioScience Taq Polymerase is delivered with 10X reaction buffer and separate MgCl₂. The enzyme is delivered with our buffer component 'Buffer-S'. The buffer is optimised for high specificity amplification of DNA-templates. Our complete buffer contains 15 mM MgCl₂. The special purification procedure guarantees Taq-Polymerase free of any DNA impurities, especially free of DNA from the conservative region of the 16S ribosomal gene.

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.

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.

Supplied buffers (alternatively with complete or incomplete buffer)

- 10 x PCR buffer with MgCl₂ : 100 mM Tris-HCl (pH 9.0 at 25°C), 500 mM KCl, 15 mM MgCl₂, 1.0% Triton X-100
- 10 x PCR buffer without MgCl₂ : 100 mM Tris-HCl (pH 9.0 at 25°C), 500 mM KCl, 1.0% Triton X-100.
- Magnesium stock solution: 25 mM MgCl₂

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 EcoRI-digested lambda DNA at 72°C in the presence of 15 - 20 units of Genaxxon Taq-DNA Polymerase.

Properties and application

The Genaxxon Taq DNA Polymerase DF is a thermostable DNA polymerase from *T. aquaticus* of high purity with good fidelity and high processivity in the DNA chain elongation reaction. Using this enzyme, amplification of DNA fragments ranging from 100 bp to 5 kbp can be achieved under standard assay conditions described below. No traces of bacterial DNA were detected in PCR Reaction with negative control and with primers complementary to the conservative region of the 16S ribosomal gene.

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Standard DNA amplification assay

Suggested Protocol using DF Taq Polymerase

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

Pipette the following into a PCR tube, mix and make up to a final volume of 50 μ L.

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.

Table 1: PCR reaction components

Components	Quantities
Template DNA	1 ng - 10 ng plasmid DNA or 5 ng - 500 ng genomic DNA
Nucleotides	1 μ L (10 mM) each of dATP, dCTP, dGTP, dTTP
10X amplification buffer	5 μ L
25 mM MgCl ₂	1.5 μ L (if no complete buffer is used)
primer 1:	4-7 μ L of 3 μ M solution (10 - 20 pmole absolute)
primer 2:	4-7 μ L of 3 μ M solution (10 - 20 pmole absolute)
sterile, bidestilled water	up to 50 μ L
Taq-Polymerase	0.25 - 1.0 μ L (1.25 - 5 units)

Table 2: MgCl₂ concentration in a 50 μ L reaction (complete buffer)

Final MgCl ₂ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM MgCl ₂ per reaction (μ L)	0	1	2	3	4	5	6

Table 3: MgCl₂ concentration in a 50 μ L reaction (incomplete buffer)

Final MgCl ₂ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM MgCl ₂ per reaction (μ L)	3	4	5	6	7	8	9

Notes:

- Drops should be collected by centrifugation and 50 μ L of mineral oil should be layered upon the reaction mixture.
- Program the thermal cycler according to the manufacturer's instructions. Each programme should start with an initial heat incubation step at 94°C for 3-5 minutes!
- Recommended elongation time is 1 minute per 1kb of target!
- For maximum yield and specificity, temperatures (annealing) and cycling times should be optimised for each new template target or primer pair.