



Genaxxon BioScience

SuperHot Taq-DNA-Polymerase

Deoxynucleosidetriphosphate: DNA nucleotidyltransferase from *Thermus aquaticus*

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Product	Cat#	Package size
Chemically modified Taq-DNA polymerase with buffer and MgCl ₂	M3307.0250	250 units
Chemically modified Taq-DNA polymerase with buffer and MgCl ₂	M3307.1000	1000 units

Product description

The Genaxxon BioScience SuperHot Taq polymerase is a modified form of thermostable DNA polymerase, which is activated by heat treatment. A chemical moiety is attached to the enzyme at the active site, which renders the enzyme inactive at room temperature. Thus, during setup and the first PCR cycle, the enzyme is not active and misprimed primers are not extended. As a result specificity and yield are increased compared to standard Taq-Polymerase. Additionally, difficult targets with high GC-content can be amplified.

Once temperature reaches optimal activating conditions, the chemical moiety is cleaved during a 15 minute heat activation step, resulting in an active Taq DNA polymerase.

Sensitivity improves multiplex PCR, an applied PCR technique that amplifies several specific targets simultaneously. Applications that previously required two or more reactions can be performed in a single reaction tube. Hence, multiplexing represents a substantial saving of time and reagents.

Key-Features

- Automated Hotstart for increased specificity and product yield
- Successful multiplex reactions
- Less formation of non-specific product
- Detection of low target copy number

Application

HotStart PCR
 PCR with high specificity (Real time PCR / quantitative PCR)
 Multiplex PCR

Storage and dilution buffer

20 mM Tris-HCl (pH 8,3), 100 mM KCl, 0,1 mM EDTA, 1 mM DTT, 50% glycerol, 0,5% Nonidet P40 and 0,5% Tween 20.

Supplied buffers (alternatively with complete or incomplete buffer)

- 10 x PCR buffer with MgCl₂ : 160 mM (NH₄)₂SO₄, 670 mM Tris-HCl pH 8.8 (at 25°C), 25 mM MgCl₂, 0.1% Tween 20
 - 10 x PCR buffer without MgCl₂ : 160 mM (NH₄)₂SO₄, 670 mM Tris-HCl pH 8.8 (at 25°C), 0.1% Tween 20
 - Magnesium stock solution: 25 mM MgCl₂

Concentration

5 units/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.



Stability

The enzyme is stable for more than 12 months if stored at -20°C.
 The enzyme is also stable for several 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 Superhot Taq-DNA Polymerase.

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Suggested Protocol using HotStart 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 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.

Table 1: PCR reaction components

Components	Quantities
Template DNA	1 ng – 10 ng plasmid DNA or 5 ng – 500 ng genomic DNA
Nucleotides	1 µl (10 mM) each of dATP, dCTP, dGTP, dTTP
10X amplification buffer	5 µl
25 mM MgCl ₂	1.5 µl (if no complete buffer is used)
primer 1:	4-7 µl of 3 µM solution (10 – 20 pmole absolute)
primer 2:	4-7 µl of 3 µM solution (10 – 20 mpole absolute)
sterile, bidistilled water	up to 50 µl
Superhot Taq-Polymerase	0.5 – 1.0 µl (2.5 - 5 units)

Table 2: MgCl₂ concentration in a 50 µl reaction

Final MgCl ₂ conc. in reaction (mM)	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Additional volume of 25 mM MgCl ₂ per reaction (µl)	0	1	2	3	4	5	6

Notes:

- Drops should be collected by centrifugation and 50 µl of mineral oil should be layered upon the reaction mixture.
- Program the thermal cycler according to the manufacturer's instructions. **Each programme must start with an initial heat activation step at 95°C for 10 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.

References:

1. Kaledin et al. (1980) Biokhimia 45, 644-651.
2. Kaledin et al. (1981) Biokhimia 46, 1576-1584.
3. Kaledin et al. (1982) Biokhimia 47, 1785-1791.

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