

Taq-Polymerase with Buffer-S (high specificity)

Deoxynucleosidetriphosphate DNA nucleotidyltransferase from *Thermus aquaticus*

fon:
+49 (0)731 - 3608 123
fax:
+49 (0)731 - 3608 962
eMail:
info@genaxxon.com
internet:
www.genaxxon.com

Product	Cat#	Package size
Taq DNA Polymerase with Buffer S and MgCl ₂	M3001.0250	250 units
Taq DNA Polymerase with Buffer S and MgCl ₂	M3001.0500	2 x 250 units
Taq DNA Polymerase with Buffer S and MgCl ₂	M3001.1000	4 x 250 units
Taq DNA Polymerase with Buffer S and MgCl ₂	M3001.2500	10 x 250 units
Taq DNA Polymerase with Buffer S and MgCl ₂	M3001.5000	20 x 250 units
Taq DNA Polymerase with Buffer S and MgCl ₂	M3001.1010	40 x 250 units

Product description

The Genaxxon bioscience Taq-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 15mM MgCl₂.

Product Specifications

Concentration:	5 units/μL
Substrate analogs:	dNTP, ddNTP, fluorescent dNTP/ddNTP
Extension rate:	2-4 kb/min. at 72°C
Half-life:	20min. at 95°C, 60min. at 94°C
5'-3' exonuclease activity:	Yes
Extra addition of A:	Yes
3'-5' exonuclease activity:	No
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

Unit definition

One unit of Taq DNA-Polymerase is defined as the amount of enzyme that incorporates 10nmol of dNTP's into acid-insoluble fraction in 30 minutes at 72°C under standard assay conditions.

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 Taq DNA-Polymerase in E-PCR buffer.
Endonuclease activity:	Plasmid DNA is incubated with Taq DNA-Polymerase in E-PCR buffer.
RNase activity:	RNA is incubated with Taq DNA-Polymerase in E-PCR buffer.
Protease activity:	Taq DNA-Polymerase is incubated in storage buffer.
Self-priming activity:	PCR is performed under standard conditions, without primers, using Taq DNA-Polymerase and human genomic DNA.

Application

Standard PCR
PCR with high specificity

fon:
+49 (0)731 - 3608 123
fax:
+49 (0)731 - 3608 962
eMail:
info@genaxxon.com
internet:
www.genaxxon.com

Supplied buffers/solutions

- 10 x PCR buffer with MgCl₂ : 100mM Tris-HCl (pH9.0 at 25 °C), 500mM KCl, 15mM MgCl₂, 1.0% Triton X-100
- 10 x PCR buffer without MgCl₂ : 100mM Tris-HCl (pH9.0 at 25 °C), 500mM KCl, 1.0% Triton X-100.
- Magnesium stock solution: 25mM MgCl₂

Stability

Genaxxon bioscience Taq-Polymerase is shipped on “blue-ice” but retain full activity at RT (15-25 °C) for at least 2 weeks.

Taq-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 Taq-Polymerase can also be stored at 2-8 °C up to 3 months.

Product Use Limitations

Taq-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

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).

PCR Protocol Part

Protocol using Taq-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. For convenience, the Mastermix (M3014) already contains a premixed solution containing HotStart Taq-Polymerase, PCR buffer, and dNTPs.

Procedure

1. **Thaw 10X buffer, dNTPs or dNTP-mix, primer solutions, and 25mM MgCl₂** (if required) 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. The optimal MgCl₂ concentration should be determined empirically but in most cases a concentration of 2.5mM, as provided in 10X E-buffer complete, will produce satisfactory results. Keep the master mix on ice.
Note: The Mg²⁺ concentration provided by the supplied PCR-buffer will produce satisfactory results in most cases. However, in some cases, reactions may be improved by increasing the final Mg²⁺ concentration according to Table 2.

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

Components	Quantities
Template DNA	< 10ng plasmid DNA < 500ng genomic DNA
Nucleotides	1µL (10mM) each dATP, dCTP, dGTP, dTTP 1µL of 10mM ready-to-use dNTP mix.
10X amplification buffer	5µL
25mM MgCl ₂ *	3µL (if no complete buffer is used)
primer 1:	0.1 - 0.5µM (5 - 25 pmol absolute)
primer 2:	0.1 - 0.5µM (5 - 25 pmol absolute)
sterile, bidestilled water	up to 50µL
Taq-Polymerase	0.25 - 0.5µL (1.25 - 2.5 units)

* if Buffer S complete is used, normally no MgCl₂ has to be added

Table 2: Final MgCl₂ concentration in a 50µL reaction

Final MgCl ₂ conc. in reaction (mM) complete buffer S	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25mM MgCl ₂ per 50µL reaction (µL)	0	1	2	3	4	5	6
Final MgCl ₂ conc. in reaction (mM) incomplete buffer S	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25mM MgCl ₂ per 50µL reaction (µL)	3	4	5	6	7	8	9

Note: The optimal Mg²⁺ concentration should be determined empirically but in most cases a concentration of 1.5mM, as provided in the 10X complete buffer S will produce satisfactory results.

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** (<1µg/reaction) 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. **When using a thermal cycler with a heated lid**, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50µL - 100µL mineral oil.
6. **Program the thermal cycler** according to the manufacturer's instructions. A typical PCR cycling program is outlined in Table 3. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

Table 3: PCR conditions (Thermal cycler)

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

7. **For simplified hot start**, proceed as described in step 8. Otherwise, place the PCR tube in the thermal cycler and start program.
8. **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 (M3006), SuperHot Taq-Polymerase (M3307) or the HotStart Mastermix (M3007).

Table 4: Recommendations for Standard PCR-Primers

Length:	18-30 nucleotides
GC-Content:	40-60%
T_m:	Design primer pairs with similar T _m values. Optimal annealing temperature may be above OR below the estimated T _m . As a starting point, use an annealing temperature of 3°C to 5°C below T _m of the primer with the lower T _m -Value.
Sequence:	Avoid complementarity 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 5: 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	