GENAXXON bioscience

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SuperHot *Taq* DNA Polymerase

chemically modified *Taq* DNA polymerase

Component	Cat#	M3307.0250	M3307.1000	M3307.5000	Colour code of cap
Chemically modified hotstart *Taq* DNA polymerase		1x 250 units	2x 500 units	10x 500 units	red
10X Buffer E complete		1x 1mL	4x 1mL	16x 1mL	orange
10X Buffer E incomplete		1x 1mL	4x 1mL	16x 1mL	yellow
25mM MgCl2		1x 1mL	1x 1mL	2x 1mL	green

Product Description

The Genaxxon bioscience SuperHot *Taq* DNA Polymerase is a chemically inactivated *Taq* DNA polymerase, which is not active before prior activation for 15 minutes at 95°C. A chemical moiety is attaché 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 cannot be extended. As a result, specificity and yield are increased compared to the standard *Taq* DNA polymerase. Additionally, difficult target with high GC-content can be denatured for a prolonged period of time without harm of the polymerase.

Once temperature reaches 95°C, the chemical moiety is cleaved during a 15-minute heat activation step, resulting in an active *Taq* DNA polymerase. This heat activation step improves sensitivity which 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.

Product Specifications

Concentration:	5 units/µL
Extension rate:	2-4 kb/min. at 72°C
Half-life:	75min. 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

Unit definition

One unit of SuperHot *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 competitor products.
PCR reproducibility:	PCR reproducibility is tested in parallel amplification reaction.
Exonuclease activity:	Linearized DNA is incubated with SuperHot *Taq* DNA Polymerase in PCR Buffer E.
Endonuclease activity:	Plasmid DNA is incubated with SuperHot *Taq* DNA Polymerase in PCR Buffer E.
RNase activity:	RNA is incubated with SuperHot *Taq* DNA Polymerase in PCR Buffer E.
Protease activity:	SuperHot *Taq* DNA Polymerase is incubated in storage buffer.
Self-priming activity: human	PCR is performed under standard conditions, without primers, using SuperHot *Taq* DNA Polymerase and genomic DNA.

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Application

Automated Hotstart PCR
PCR with high specificity (Real time PCR / quantitative PCR)
Detection of low target copy number
2-step RT-PCR

Supplied buffers/solutions

- *10X PCR buffer with Ammonium sulphate and with MgCl2*

- *10X PCR buffer with Ammonium sulphate but without MgCl2*

- *Magnesium stock solution*: 25mM MgCl2

Stability

The Genaxxon bioscience SuperHot *Taq* DNA Polymerase is shipped on wet ice but retain full activity at RT (+15°C to +25°C) for at least 2 weeks.

The Genaxxon bioscience SuperHot *Taq* DNA Polymerase, including buffers, 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.

Alternatively, the Genaxxon bioscience SuperHot *Taq* DNA Polymerase can also be stored at +2 to +8°C for at least 8-10 months without loss of activity if not opened.

Product Use Limitations

The SuperHot *Taq* DNA 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).

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PCR Protocol Part

Protocol using SuperHot *Taq* DNA 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.

Points 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.

Procedure

1. **Thaw 10X buffer, dNTPs or dNTP-mix, primer solutions, and 25mM MgCl2 (if required) at RT or on ice.**
   Keep solutions on ice after complete thawing and mix well before use to avoid local differences in salt concentrations.

   Optional: Prepare a primer mix of an appropriate concentration using sterile, bidest water. This is recommended if several amplification reactions using the same primer pair are to be performed. The final volume of diluted primer mix plus the template DNA, added at step 4, should not exceed 12.5µL per reaction.

2. **Prepare a master mix according to Table 1**
   A master mix typically contains all 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.

   Note: A negative control (PCR without template DNA) should be included in every experiment.

   Note: It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.

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

Components	Quantities for a 50µL PCR reaction
SuperHotStart *Taq* DNA Polymerase	0.25 – 0.5µL (1.25 – 2.5 units)
10X PCR buffer	5µL
Nucleotides	1µL (10mM) each dATP, dCTP, dGTP, dTTP or 1µL of 10mM ready-to-use dNTP mix.
25mM MgCl2* (optional)	5µL (if no complete buffer is used)
primer 1:	0.5µL (0.25 – 2.5µL) 0.1µM (0.05 - 0.5µM (5 – 25 pmol absolute))
primer 2:	0.5µL (0.25 – 2.5µL) 0.1µM (0.05 - 0.5µM (5 – 25 pmol absolute))
Probe (10µM) ** optional	x µL (0.05 – 1µM)
Template DNA	<10ng plasmid DNA or <500ng genomic DNA
sterile, bidestilled water	up to 50µL

* if Buffer E complete is used, normally no MgCl2 has to be added.
** The necessary concentration of probe depends very much on the probe sequence and the kind of probe. Please test for optimum!

Table 2: Final MgCl2 concentration in a **50µL reaction**

Final MgCl2 conc. in reaction (mM) **complete buffer E**	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Additional volume of 25mM MgCl2 per 50µL reaction (µL)	0	1	2	3	4	5	6

Final MgCl2 conc. in reaction (mM) **incomplete buffer E**	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25mM MgCl2 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 2.5mM, as provided in the 10X complete buffer E will produce satisfactory results.

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1. **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.
2. **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.
3. **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.
4. **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.
5. **Place PCR tubes in the thermal cycler and start program.**

Table 3: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	15 min. *	95°C	it is very important to denature for 15 minutes!
3-step cycling			
Denaturation:	0.5 - 1 min.	95°C	
Annealing:	0.5 - 1 min.	50 - 68°C	Approximately 5°C below Tm of primers.
Extension:	0.5 - 1 min.	72°C	For PCR products longer than 1kb, use an extension time of approximately 1min./kb DNA.
Number of Cycles	25 - 35		
Final extension	5 min.	72°C	optional - not necessary

* It is very important to have at least a 10-minute activation step at 95°C. Otherwise the polymerase will not work fully! We recommend programming a 15-minute activation step to get full recovery of the polymerase activity.

Note: After amplification, samples can be stored at +2°C to +8°C overnight, or -20°C for long term storage.

Table 4: Recommendations for Standard PCR-Primers

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