GENAXXON bioscience
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SuperHot Mastermix (2X)

with chemically modified *Taq* DNA polymerase

Component	Cat#	M3007.0100	M3007.0500	Colour code of cap
Premixed 2-time Mastermix for real time PCR containing chemically modified *Taq* DNA polymerase for hotstart PCR		2x 1.25mL 200 reactions	10x 1.25mL 1000 reactions	blue
25mM MgCl2		1x 1mL	1x 1mL	green

Product Description

The Genaxxon bioscience SuperHot Mastermix (2X) is a ready-to-use PCR mixture of the Genaxxon chemically inactivated *Taq* DNA Polymerase (M3307), special PCR buffer, $MgCl_2$, dNTPs and additives optimized for use in Real-Time PCR with probes (dual labeled oligos, like TaqMan® Probes, Molecular Beacons, etc.). The 2X mix contains all components for PCR, except DNA template and primers. The mixture was tested against products from market leaders and has shown to be highly reliable for RealTime PCR in Real-Time PCR Block Thermal Cyclers. Difficult targets with high GC-content can be amplified.

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.

The SuperHot Mastermix is shipped with additional MgCl2 solution. The small and convenient aliquot size of 1.25mL ensures and secures safe handling.

Product Specifications

Concentration:	2 time ready-to-use master mix
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 used for the Genaxxon bioscience SuperHot Mastermix 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 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

- *Magnesium stock solution*: 25mM MgCl2

Stability

The Genaxxon bioscience SuperHot Mastermix is shipped on wet ice but retain full activity at RT (+15°C to +25°C) for at least 1 week.

The Genaxxon bioscience SuperHot Mastermix, 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.

Alternatively the Genaxxon bioscience SuperHot Mastermix 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 Mastermix 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).

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com)

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PCR Protocol Part

Protocol using SuperHot Mastermix (2X)

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

- SuperHot Mastermix provides a final concentration of 1.5mM MgCl2 which will produce satisfactory results in most cases. However, if a higher Mg2+ concentration is required, the Genaxxon bioscience SuperHot Mastermix is shipped with additional 25mM MgCl2.
- 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.

Procedure

1. **Thaw primer solutions**
   Keep on ice after complete thawing, and mix well before use.

   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. **Thaw SuperHot Mastermix (2X)** at RT or on ice.
   Keep the solutions on ice after complete thawing. It is very important to mix the SuperHot Mastermix well before use to avoid local differences in salt concentration. The Genaxxon bioscience SuperHot Mastermix is provided as a 2X concentrated (i.e. a 12.5µL volume of SuperHot Mastermix is required for PCR reactions with a final volume of 25µL). For volumes smaller than 50µL, the 1:1 ratio of SuperHot Mastermix to diluted primer mix, template DNA and water should be maintained. A negative control (PCR without template DNA) should be included in every experiment. It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.
3. **Distribute the appropriate volume of diluted primer mix into the PCR tubes containing the SuperHot Mastermix.**
4. **Add template DNA** (<1µg/reaction) to the individual PCR tubes.
   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of final PCR volume.

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

Components	Quantities
SuperHot Mastermix	12.5mL
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))
Template DNA	variable volume: < 5ng plasmid DNA variable volume: < 250ng genomic DNA
sterile, bidestilled water	up to 25µL

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

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 mineral oil.
6. **Program the thermal cycler** according to the manufacturer's instructions.
   A typical PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.
7. **Place PCR tubes in the thermal cycler and start program.**

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Table 2: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	15 min *	95°C	highly recommended not to go below 10 minutes initial denaturation.
3-step cycling			
Denaturation:	0.5 - 1 min.	95°C	
Annealing:	0.5 - 1 min.	50 - 68°C	Approximately 5°C* below lower 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	30 - 40		
Final extension	5 min.	72°C	

* It is very important to have at least a 10 minutes 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 3: 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 4: 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	

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