GENAXXON bioscience
Tel: +49(0)731 – 3608 123
Fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

SuperHot Multiplex MasterMix (2X)

with chemically modified (inhibited) *Taq* DNA polymerase

Component	Cat#	M3244.0100	M3244.0500	Colour code of cap
Premixed PCR master mix optimized for Multiplex-PCR.		2 x 1.25mL	10 x 1.25mL	green

Product Description

Multiplex PCR is a method that enables amplification of two or more amplicons simultaneously in a single reaction tube/reaction. It is widely used in genotyping and different areas of DNA testing in research, forensic and diagnostic laboratories.

The Genaxxon bioscience SuperHot Multiplex MasterMix (2X) is an all-in-one 2x master mix with SuperHot Taq DNA polymerase, in an optimized buffer system with dNTPs and MgCl2. Simply add primers, template and water to successfully carry out multiplex PCR.

SuperHot Taq DNA Polymerase is a chemically modified form of standard Taq DNA polymerase, which is activated by heat treatment. The 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 ramp of thermal cycling, the enzyme is not active and misprimed primers are not extended. The result is higher specificity, increased sensitivity and greater yields when compared to standard DNA polymerases, making this enzyme especially well-suited for multiplex PCR.

Product Specifications

Concentration:	2 time ready-to-use master mix
Extension rate:	4-6 kb/min. at 72°C
Half-life:	10min. at 97°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

Unit definition

One unit of SuperHot Taq DNA-Polymerase used for the Genaxxon bioscience SuperHot Multiplex HS Mastermix (2X) 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 Multiplex HS PCR buffer.
Endonuclease activity:	Plasmid DNA is incubated with SuperHot Taq DNA polymerase in Multiplex HS PCR buffer.
RNase activity:	RNA is incubated with SuperHot Taq DNA polymerase in Multiplex HS PCR buffer.
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.

GENAXXON bioscience GmbH
Söflinger Str. 70 ● 89077 Ulm ● Geschäftsführer: Dr. Norbert Tröndle
Amtsgericht: 89014 Ulm ● HRB 641623
U.St.-Id.Nr. DE 220 603 767 ● Deutsche St.-Nr. 88002/33658

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www.genaxxon.com

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Application

Automated hot start PCR
Gene expression
Multiplex qPCR / Multiplex PCR with fluorochromes
Genotyping
Copy number analysis

Stability

The Genaxxon bioscience SuperHot Multiplex Mastermix (2X) is shipped on wet ice but retain full activity at RT (+15 - +25°C) for at least 3-4 days.

The Genaxxon bioscience SuperHot Multiplex Mastermix (2X), 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 SuperHot Multiplex Mastermix (2X) can also be stored at +2°C to +8°C°C up to 3 months.

Product Use Limitations

The SuperHot Multiplex Mastermix (2X) 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 Multiplex 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

- All primers should have the same melting temperature.
- Use equal concentrations of all primers for the initial test.
- If one product gives a much stronger band than the others, reduce the primer concentration for this target.
- If one product gives a much weaker band than the others, increase the primer concentration of this target.
- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis. Working on ice is not required.
- The MgCl2 concentration in the final reaction is 3mM. In some applications, more MgCl2 is required for best results.
- 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 nuclease-free 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 Multiplex Mastermix (2X)** at RT or on ice.
   It is very important to mix the SuperHot Multiplex Mastermix (2X) well before use to avoid local differences in salt concentration. Spin vials briefly after mixing. The Genaxxon bioscience SuperHot Multiplex Mastermix (2X) is provided as a 2X concentrated (i.e., a 10µL volume of SuperHot Multiplex Mastermix (2X) is required for PCR reactions with a final volume of 20µL). For volumes smaller than 20µL, the 1:1 ratio of SuperHot Multiplex Mastermix (2X) to diluted primer mix, template DNA and water should be maintained. A negative control (PCR without template DNA) should be included in every experiment.

3. **Distribute the appropriate volume of diluted primer mix into the PCR tubes containing the SuperHot Multiplex Mastermix (2X).**

4. **Add template DNA** to the individual PCR tubes containing the reaction mix.

Table 1: PCR reaction components using SuperHot Multiplex Mastermix (2X) (**20µL PCR reaction**)

Components	Quantities/Volumes	Final concentrations/amount
SuperHot Multiplex Mastermix (2X):	10µL	1x
each forward primer (10µM):	variable, e.g. 2µL	0.1 – 0.5µM
each reverse primer (10µM):	variable, e.g. 2µL	0.1 – 0.5µM
Probe	variable, e.g. 2µL	0.2 – 0.5µM
Template DNA	variable	genomic DNA: 1 – 20ng plasmid DNA: 1 – 50pg bacterial DNA: 0.1 – 1ng
Nuclease-free water	adjust to 20µL	
Total volume	**20µL**	

The table above shows the suggested starting conditions. Please adopt the protocol to your needs.
Theoretically usable conditions in brackets.

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

Table 2: PCR conditions (**Thermal cycler**) – 3-step PCR protocol

Step	time	temperature	comments
Initial denaturation:	15 min.	95°C	
Denaturation:	5 - 10 seconds	95°C	
Annealing:	5 - 10 seconds	50 – 68°C	Approximately 3 - 5°C below Tm of primers.
Extension:	5 - 10 seconds	72°C	For PCR products longer than 1kb, use an extension time of approximately 1min./kb DNA.
Number of Cycles	30 – 45		
Final extension	5 min.	72°C	Occasionally used

Table 3: PCR conditions (**Thermal cycler**) – 2-step PCR protocol

Step	time	temperature	comments
Initial denaturation:	15 min.	95°C	
Denaturation:	5 - 10 seconds	95°C	
Annealing/Extension combined	5 - 10 seconds	60 – 68°C	Depends on primers. Approximately 3 - 5°C below Tm of primers.
Number of Cycles	30 – 45		
Final extension	5 min.	72°C	Occasionally used

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

Note: For maximum efficiency and specificity, annealing temperatures, as well as extension time, primer/probe concentration and template DNA concentration may need to be optimized.

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	