GENAXXON bioscience

fon: +49(0)731 – 3608 123
fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

RedMastermix Hot (2x)

with an inhibited *Taq* DNA polymerase and a red tracking dye

Product	Cat#	Package size
Premixed RedMastermix Hot (2x)with an inhibited *Taq* DNA polymerase and a red tracking dye.	M3221.0100	2 x 1mL
Premixed RedMastermix Hot (2x) with an inhibited *Taq* DNA polymerase and a red tracking dye.	M3221.0500	10 x 1mL

Product Description

The Genaxxon bioscience RedMastermix Hot (2x) with an inhibited *Taq* DNA polymerase is a ready-to-use PCR mixture of the Genaxxon inactivated *Taq* DNA polymerase (M3001, special PCR buffer, $MgCl_2$, and dNTPs The 2X mix contains all components for PCR, except DNA template and primers.

Genaxxon's RedMastermix Hot (2x) has been developed for the sensitive detection of low-copy templates as well as for efficient and, above all, specific amplification of complex templates such as GC- or AT-rich sequences.
The master mix contains a red dye that allows direct application of the PCR product onto the electrophoresis gel, with the red dye enabling monitoring of the gel run. Compared to standard Taq PCR master mixes, the optimized buffer mixture with MgCl2 and dNTP results in an increased yield of amplicons up to 6kb even under fast PCR conditions (up to 2kb).

Highly specific Hotstart Mastermix for routine and diagnostic applications. Fast amplification up to 2kb. Ready-to-use, consisting of:

- inhibited Taq DNA Polymerase
- optimized PCR-Puffer
- dNTPs
- $MgCl_2$

for your HotStart PCR.

Ready-to-use RedMastermix Hot (2x). All you have to do is add the primers and the template DNA. The special composition of the buffer guarantees reproducible results even after repeated thawing and freezing cycles. The RedMastermix Hot (2x) will be sent in 1mL aliquots.

The aptamer inhibited Taq Polymerase does not need a prolonged activation step. You can just program a initial denaturing temperature of 2 minutes.

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 HotStart *Taq* DNA polymerase used for the Genaxxon bioscience HotStart *Taq* 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.

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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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 RedMastermix Hot (2x).
Endonuclease activity:	Plasmid DNA is incubated with RedMastermix Hot (2x).
RNase activity:	RNA is incubated with RedMastermix Hot (2x).
Protease activity:	RedMastermix Hot (2x) is incubated in storage buffer.
Self-priming activity:	PCR is performed under standard conditions, without primers, using RedMastermix Hot (2x) and human genomic DNA.

Application

Automated Hotstart PCR
PCR with high specificity
Detection of low target copy number

Stability

The Genaxxon bioscience RedMastermix Hot (2x) is shipped on wet ice but retain full activity at RT (+15°C to +25°C) for at least 1 week.

The Genaxxon bioscience RedMastermix Hot (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.

Product Use Limitations

The RedMastermix Hot (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).

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 RedMastermix Hot (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.

- RedMastermix Hot (2x) provides an optimized concentration of MgCl2 which will produce satisfactory results in most cases.
- 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, demineralized 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 RedMastermix Hot (2x) at RT or on ice.**
   Keep the solutions on ice after complete thawing. It is very important to mix the RedMastermix Hot (2x)well before use to avoid local differences in salt concentration. The Genaxxon bioscience RedMastermix Hot (2x) is provided as a 2X concentrated (i.e. a 10µL volume of RedMastermix Hot (2x) is required for PCR reactions with a final volume of 20µL). For volumes smaller than 20µL, the 1:1 ratio of RedMastermix Hot (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. 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 RedMastermix Hot (2x).
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 (**20µL PCR reaction**)

Components	Quantities
RedMastermix Hot (2x)	10µL
primer 1: primer 2: Template DNA	0.4µL (0.25 – 2.5µL) 0.1µM (0.05 - 0.5µM (5 – 25 pmol absolute)) 0.4µL (0.25 – 2.5µL) 0.1µM (0.05 - 0.5µM (5 – 25 pmol absolute)) variable volume: < 5ng plasmid DNA variable volume: < 250ng genomic DNA
sterile, bidestilled water	up to 20µL

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5. **When using a thermal cycler with a heated lid**, do not use mineral oil. Proceed directly to step 6.
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:	2 to 4 min *	94°C	4 minutes are highly recommended in case of difficult target (e.g., high GC content).
3-step cycling			
Denaturation:	30 seconds	94°C	
Annealing:	30 seconds	50 - 68°C	Approximately 5°C* below lower Tm of primers.
Extension:	0.5 - 1 min.	72°C	program 1 minute extension time per 1kb template.
Number of Cycles	30 - 40		
Final extension	5 min.	72°C	

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

Analyse the amplification reaction by gel electrophoresis using an acrylamide or agarose gel of appropriate percentage.

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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