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GENAXXON bioscience

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GENAXXON bioscience

GreenMasterMix (2X)

No ROX™

qPCR master mix with fluorescence dye
without passive reference dye

suited for example for following instruments:

**BioRad CFX96 Touch™, CFX384 Touch™, CFX Connect™,
DNA Engine Opticon® 2, Chromo4™, iCycler iQ™ and My iQ™ ,
Roche LightCycler® 480, LightCycler® 1536, LightCycler® Nano,
LightCycler® 96 and QuantStudio™ instruments, Thermo Scientific™
PikoReal™, Cepheid SmartCycler®, Bio Molecular Systems Mic qPCR cycler,
Qiagen Rotor Gene Q, Rotor Gene 6000, MyGo Mini and MyGo Pro.**

Cat#: M3023

Version: 221104

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

We Aim for Your Success.

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Related Products

Cat #	Description
M3011	GreenMastermix Low ROX™ for real time PCR / qPCR.
M3023	GreenMastermix without ROX™ for real time PCR / qPCR.
M3052	GreenMastermix with High ROX™ for real time PCR / qPCR.
M3010	ProbeMastermix with High ROX™ for real time PCR / qPCR.
M3031	ProbeMastermix with Low ROX™ for real time PCR / qPCR.
M3045	ProbeMastermix without ROX™ for real time PCR / qPCR.
M3307	SuperHotStart *Taq* DNA Polymerase for real time PCR / qPCR / multiplex PCR.
M3006	HotStart *Taq* DNA Polymerase with antibody for real time PCR / qPCR / multiplex PCR.
M3001	*Taq* DNA polymerase with Buffer S for high specifity PCR.
M3043	*Taq* DNA polymerase with Buffer E for high efficiency PCR.
M3014	Mastermix with *Taq* DNA polymerase for high efficiency PCR.
M3029	Mastermix with *Taq* DNA polymerase and a red dye for visualizing pipetting.
M3002	*Pwo* Polymerase for proof reading PCR.
M3004	Pfunds Polymerase for proof reading PCR.
M3003	ReproFast Polymerase high efficiency proof reading PCR (up to 7kb).
M3012	ReproHot/KOD DNA Polymerase high efficiency proof reading PCR (up to 7kb) and hotstart conditions.
M3009	SNP Pol DNA Polymerase optimized for detection of single point mutations.
M3025	SNP PolTaq DNA Polymerase optimized for detection of single point mutations with probes like TaqMan, Beacons, etc..

Table 5: 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 6: 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	

"Your success is our aim"

For more information: www.genaxxon.com

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Table 3: PCR conditions - Three step program (Thermal cycler)

Step	time	temperature	comments
Initial denaturation:	15 min.	95°C	it is important to apply 15 minutes 95°C!
3-step cycling			
Denaturation:	15 - 30 sec. *	95°C	
Annealing:	15 - 30 sec. *	55°C - 65°C	Approximately 3°C below Tm of primers.
Extension:	15 - 30 sec. *	72°C	
Number of Cycles	25 - 35		For low copy number genes, it might be necessary to use cycle number of up to 45.

Note: After amplification, samples can be stored at +2°C to +8°C overnight, or -20°C for long term storage.
Note: Denaturation and Annealing/Extention times can vary between thermocyclers and qPCR master mixes!

Table 4: PCR conditions - Two step program (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	15 min.	95°C	it is important to apply 15 minutes 95°C!
2-step cycling			
Denaturation:	15 - 30 sec. *	95°C	
Annealing:	30 - 60 sec. *	55°C - 65°C	Approximately 3°C below Tm of primers.
Number of Cycles	25 - 35		For low copy number genes, it might be necessary to use cycle number of up to 45.

Note: After amplification, samples can be stored at +2°C to +8°C overnight, or -20°C for long term storage.
Note: Denaturation and Annealing/Extension times can vary between thermocyclers and qPCR master mixes!

This Genaxxon GreenMastermix without ROX™ is designed for example for the following instruments:

**BioRad CFX96 Touch™, CFX384 Touch™, CFX Connect™,
DNA Engine Opticon® 2, Chromo4™, iCycler iQ™ and My iQ™ ,
Roche LightCycler® 480, LightCycler® 1536, LightCycler® Nano,
LightCycler® 96 and QuantStudio™ instruments, Thermo Scientific™ PikoReal™, Cepheid
SmartCycler®, Bio Molecular Systems Mic qPCR cycler,
Qiagen Rotor Gene Q, Rotor Gene 6000, MyGo Mini and MyGo Pro.**

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GENAXXON bioscience

Manual Contents

GENAXXON bioscience GmbH
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Amtsgericht: 89014 Ulm ● HRB 641623
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Product	Cat#	Package size
Premixed Mastermix for real time PCR / qPCR with green fluorescence dye, without **ROX™**: **Mix for 200 x 25µL reaction volume.**	M3023.0100	2 x 1.25mL (200 x 25 µL reactions)
Premixed Mastermix for real time PCR / qPCR with green fluorescence dye, without **ROX™**: **Mix for 1000 x 25µL reaction volume.**	M3023.0500	10 x 1.25mL (1000 x 25µL reactions)

Introduction

Quantitative PCR is an important tool for SNP and gene expression analysis. This GreenMasterMix is ideal for most quantitative experiments/applications. This mastermix has been designed to perform on real-time instruments that require no ROX™ as internal reference dye, e.g., the **Rotor-Gene™** from Qiagen or the **Roche LightCycler® 480** from Hofmann La-Roche.

Product Description

The Genaxxon GreenMasterMix (2X) is a ready-to-use PCR mixture that contains our chemically modified SuperHotStart *Taq* DNA Polymerase (M3307), which improves PCR amplification by decreasing background from non-specific amplification and increasing amplification of the desired product(s). Furthermore, SuperHotStart *Taq* DNA Polymerase is inactive at room temperature thus eliminating the necessity of working on ice during experiment set-up. The GreenMasterMix without ROX™ contains special PCR buffer, $MgCl_2$, dNTPs and a green fluorescence dye, and additives optimized for use in real time PCR with an unspecific fluorescence dye. This 2X mix can successfully amplify and detect a variety of DNA targets such as cDNA, genomic and plasmid DNA with a detection limit down to 6 copies per PCR reaction. The small and convenient aliquot size of 1.25mL ensures and secures safe handling.

Once temperature reaches 95°C, the chemical modification is deactivated, resulting in an active *Taq* DNA polymerase. This activation step needs at least 10 minutes to be effective. The 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 small and convenient aliquot size of 1.25mL ensures and secures safe handling.

High Efficiency and Specificity

To examine the efficiency of the GreenMasterMix No ROX™, a 4-fold dilution series with gDNA was set up for PAH target. Samples were made in triplicates from 80ng gDNA per well, down to 80pg gDNA per well.

The result depicted in figure 1 show high precision and efficiencies close to 100%.

The identical melting curves show a high specificity of the product, and the standard curve shows linear detection range and a high accuracy of the Genaxxon GreenMasterMix, respective the Genaxxon SuperHotStart *Taq* DNA Polymerase (M3307) (figure 1).

This product is for research use only.

Table 1: PCR reaction components using GreenMastermix (2X) (**25µL PCR reaction**)

Components	Quantities	Final concentration
GreenMastermix	12.5µL	1X
primer 1 (10µM)	0.5µL (0.25 – 2.5µL)*	0.1µM (0.05 – 0.5µM)**
primer 2 (10µM)	0.5µL (0.25 – 2.5µL)*	0.1µM (0.05 – 0.5µM)**
Template DNA	x µL (variable volume)	genomic DNA: 20ng (1 – 100ng)** plasmid DNA: 0.5ng (0.1 – 1ng)** bacterial DNA: 5ng (1 – 10ng)**
nuclease free water	up to 25µL	

* Suggested starting conditions (Optimization of primer concentration is highly recommended)
** Theoretically used conditions in brackets.

5. **Gently mix** without creating bubbles* (do not vortex). * Bubbles interfere with detection of fluorescence.
6. **When using a thermal cycler with a heated lid**, do not use mineral oil. Proceed directly to step 7. Otherwise, overlay with approximately 50µL mineral oil.
7. **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.
8. **Place PCR tubes in the thermal cycler and start program.**

PCR Protocol Part

Protocol using Genaxxon GreenMastermix (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

- For the highest efficiency in real time PCR using dual labelled probes, targets should be in the range of 90 – 250bp in length.
- Readjust threshold value for analysis of every run.
- GreenMastermix 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, 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 GreenMastermix (2X)** at RT or on ice.
   Keep the solutions on ice after complete thawing. It is very important to mix the GreenMastermix well before use to avoid local differences in salt concentration. The Genaxxon bioscience GreenMastermix is provided as a 2X concentrated (i.e., a 12.5µL volume of GreenMastermix is required for PCR reactions with a final volume of 25µL). For volumes smaller than 50µL, the 1:1 ratio of GreenMastermix 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 GreenMastermix.**
4. **Add template DNA** 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.

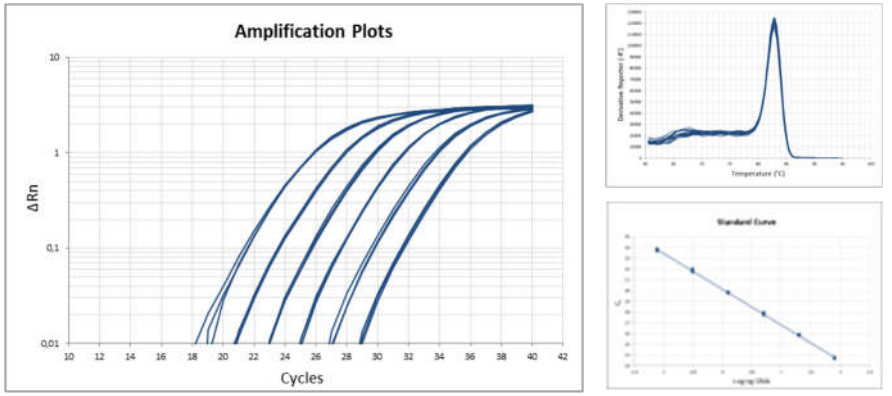

Figure 1: Amplification plot of a 4-fold dilution series for PAH target amplified from human gDNA. Starting amounts of 80ng gDNA, amplified in triplicates using GreenMasterMix (2X) No ROX™.

Stability of PCR mix at room temperature!

Due to the stability of our GreenMasterMix No ROX™ and the inactivity of the SuperHotStart *Taq* DNA Polymerase the mix allows scientists to pre-assemble the qPCR reaction(s), store it, and run it several hours later, when convenient.

A pre-assembled qPCR plate was incubated in darkness at room temperature (20°C – 25°C) for 72 hours and run at the Rotor-Gene™ from Qiagen together with a newly mixed reaction setup. The results showed similar C_t values with very low standard deviation (figure 2), thus confirming stability of our GreenMasterMix.

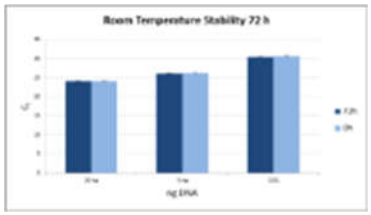

Figure 2: A PCR plate was pre-assembled for qPCR reaction and incubated in darkness at room temperature for 72 hours. The result shows high stability and inactivation of the SuperHotStart *Taq* DNA Polymerase before hot start.

Detection limit

Detection limit down to 6 copies was reached with our GreenMasterMix (2X) No ROX™.

Compatibility

GreenMasterMix (2X) No ROX™ is designed for real-time instruments that require no ROX™ as internal reference dye, e.g., Rotor-Gene™ from Qiagen.

Reproducibility

GreenMasterMix (2X) No ROX™ produces reliable and reproducible results. A demonstration of 80 replicates, containing GreenMasterMix (2X) No ROX™ and 20ng gDNA per well, was run on the Rotor-Gene™ instrument from Qiagen. The results showed close to identical amplification curves and a standard deviation of 0.084 (figure 3).

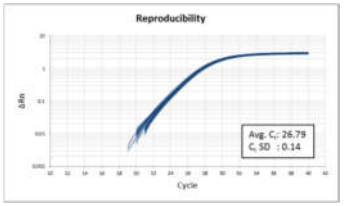

Figure 3: 80 replicates of the GreenMasterMix (2X) No ROX™ and 20ng gDNA, show a standard deviation of only 0.14.

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 SuperHotStart Taq DNA-Polymerase in PCR buffer E.

Endonuclease activity: Plasmid DNA is incubated with SuperHotStart Taq DNA-Polymerase in PCR buffer E.

RNase activity: RNA is incubated with SuperHotStart Taq DNA-Polymerase in PCR buffer E.

Protease activity: SuperHotStart Taq DNA-Polymerase is incubated in storage buffer.

Self-priming activity: PCR is performed under standard conditions, without primers, using SuperHotStart Taq DNA-Polymerase and human genomic DNA.

Unit definition

One unit of SuperHotStart Taq DNA-Polymerase used for the Genaxxon bioscience GreenMastermix 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.

Application

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

Features

- All-in-one optimized master mix, including green dye
- High sensitivity
- High efficiency and high specificity
- Wide dynamic range
- High reproducibility
- Hot start capacity for room temperature setup (no pipetting on ice necessary)

Stability

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

The Genaxxon bioscience GreenMastermix, 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.

All Genaxxon bioscience realtime master mixes can also be stored unopened at +2 to +8°C up to 10 months without loss of activity.

Product Use Limitations

GreenMastermix 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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