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GENAXXON bioscience

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ProbeMasterMix FAST
Low ROX™

qPCR master mix without fluorescence dye
with 50nM passive reference dye for FAST qPCR

suited for example for following instruments:

Applied Biosystems® 7500, 7500 Fast and ViiA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx.

Cat#: M3152

Version: 220622

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Component	Cat#	M3152.0200	M3152.1000	Colour code of cap and tube
ProbeMasterMix FAST Low ROX™ premixed qPCR master mix without green fluorescence dye, with 50nM ROX for real time PCR / qPCR.		2 x 1mL (200 x 25µL reactions)	10 x 1mL (1000 x 25µL reactions)	amber

Introduction

Quantitative PCR is an important tool for SNP and gene expression analysis. This ProbeMasterMix is ideal for most quantitative experiments/applications. The ProbeMasterMix FAST Low ROX™ has been designed to perform on real-time instruments that require Low ROX™ (50nM) as internal reference dye, e.g., QuantStudio™ instruments from ThermoFisher.

Product Description

The ProbeMasterMix FAST Low ROX™ with an inhibited *Taq* DNA polymerase for hotstart PCR is an optimized ready-to-use mixture that can be used for realtime PCR (qPCR) with all standard qPCR machines.

The Genaxxon ProbeMasterMix FAST Low ROX™ is a ready-to-use PCR mixture that contains a modified HotStart *Taq* DNA polymerase (M3006), which improves PCR amplification by decreasing background from non-specific amplification and increasing amplification of the desired product(s). Furthermore, HotStart *Taq* DNA polymerase is inactive at room temperature thus eliminating the necessity of working on ice during experiment set-up. The ProbeMasterMix FAST Low ROX™ contains a special PCR buffer, $MgCl_2$, dNTPs, and additives optimized for use in real time PCR with specific probes.

Once temperature reaches >70°C, the inhibition is deactivated, resulting in an active *Taq* DNA polymerase. This activation step needs no additional time to be effective. Even 1 second will be enough! The heat activation step improves sensitivity which improves multiplex PCR, an applied PCR technique that amplifies several specific targets simultaneously.

ProbeMasterMix FAST Low ROX™ is able to amplify PCR products up to 4 kb with genomic DNA and up to 6 kb with Lambda DNA, and is appropriate for use with pure DNA solutions, cDNA, and bacterial colonies as templates. The HotStart *Taq* polymerase included in this master mix possesses 5'->3' polymerase- as well as a 5'-flap endonuclease activity and generates 3'dA (adenine) overhang which may well be used for TA-cloning with a detection limit down to 6 copies per PCR reaction.

The small and convenient aliquot size of 1mL ensures and secures safe handling.

This product is for research use only.

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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 specificity 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	*Pfu* 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..

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PCR Protocol Part

Protocol using Genaxxon ProbeMasterMix FAST Low ROX™.

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.
- **ProbeMasterMix FAST Low ROX™** 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 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 ProbeMasterMix FAST Low ROX™** at RT or on ice, invert the tube 6-8 times. Do not vortex as this may damage the enzyme.
   Keep the solutions on ice after complete thawing. It is very important to mix the ProbeMasterMix FAST Low ROX™ well before use to avoid local differences in salt concentration.
3. The Genaxxon bioscience **ProbeMasterMix FAST Low ROX™.** is provided as a 2X concentrated (i.e., a 10µL volume of **ProbeMasterMix FAST Low ROX™** is required for PCR reactions with a final volume of 20µL). For volumes smaller than 20µL, the 1:1 ratio of **ProbeMasterMix FAST Low ROX™** to diluted primer mix, template DNA and water should be maintained.
4. **Distribute the appropriate volume** of diluted primer mix into the PCR tubes containing the **ProbeMasterMix FAST Low ROX™.**
5. **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.

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.

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Table 1: PCR reaction components using ProbeMasterMix FAST (**20µL PCR reaction**)

Components	Quantities	Final concentration
ProbeMasterMix FAST Low ROX™	10	1X
primer 1:	variable (e.g.: 1µL)	200nM (100-400nM recommended)*
primer 2:	variable (e.g.: 1µL)	200nM (100-400nM recommended)*
Template DNA	<5ng plasmid DNA or <250ng genomic DNA	
nuclease free water	adjust to 20µL	

* Suggested starting conditions (Optimization of primer concentration is highly recommended)

6. **Gently mix** without creating bubbles* (do not vortex). * Bubbles interfere with detection of fluorescence.
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.**

Application

- Automated Hotstart 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 for fast qPCR
- High sensitivity
- High efficiency and high specificity
- Wide dynamic range
- High reproducibility
- Hot start capacity for room temperature setup (no pipetting on ice necessary)

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Detection limit

Detection limit down to 6 copies was reached with our ProbeMasterMix FAST Low ROX™.

Compatibility

ProbeMasterMix FAST Low ROX™.is designed for real-time instruments that require a low concentration of ROX™ as internal reference dye, e.g. QuantStudio™ instruments from ThermoFisher.

Quality control

Genaxxon bioscience ProbeMasterMix FAST Low ROX™ undergoes stringent quality controls. Each lot is tested in a probe-based qPCR with cDNA and lambda DNA input.

The enzyme purity and homogeneity of >98% is validated.

All master mixes are free of endonuclease- and exonuclease activity.:

- Incubation of 1 µg plasmid DNA with 5 U for 4h at 37°C and 72°C
- Incubation of 1 µg of a DNA size standard with 5 U for 4h at 37°C and 72°C

For more information: www.genaxxon.com

Standard PCR-Protocol

Standard cycling conditions can be applied for the majority of qPCR assays. However, cycling conditions highly depend on the primer, probe, amplicon and input material and thus might require adjustments.

Table 2: Standard PCR conditions - 2-step PCR protocol (Thermal cycler)

Step	time	temperature	comments
Initial Denaturation:	1 - 3 min	92°C -95°C	
Denaturation:	5 - 10 sec	92°C -95°C	
Annealing/Extension:	10 - sec	60°C*	*depending on primer, approx. 5°C below lower Tm of primers
Number of Cycles	25 - 40		

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

Table 3: Standard PCR conditions - 3-step PCR protocol (Thermal cycler)

Step	time	temperature	comments
Initial Denaturation:	1 - 3 min	92°C -95°C	
Denaturation:	5 - 10 sec	92°C -95°C	
Annealing:	1 - 5 sec	60°C	depending on primer, approx. 5°C below lower Tm of primers
Extension:	10 - 20 sec	72°C	
Number of Cycles	25 - 40		

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

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Ultra-Fast Protocol

Ultra-fast cycling conditions can be applied for the majority of qPCR assays, provided that your primer/probe sets do not show unspecific binding. Please not that ultra-fast cycling conditions highly depend on the ramping rate of your qPCR cycler, primer, probe, amplicon and input material and thus might require adjustments.

Table 4: Ultra-fast PCR conditions - 2-step PCR protocol (Thermal cycler)

Step	time	temperature	comments
Initial Denaturation:	1 min	92°C -95°C	
Denaturation:	1 sec	92°C -95°C	
Annealing/Extension:	1 - 5 sec	60°C*	*depending on primer, approx. 5°C below lower Tm of primers
Number of Cycles	25 - 40		

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

Table 5: Ultra-fast PCR conditions - 3-step PCR protocol (Thermal cycler)

Step	time	temperature	comments
Initial Denaturation:	1 min	92°C -95°C	
Denaturation:	1 - 5 sec	92°C -95°C	
Annealing:	1 - 5 sec	60°C	depending on primer, approx. 5°C below lower Tm of primers
Extension:	1 sec	72°C	
Number of Cycles	25 - 40		

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

Stability

The Genaxxon bioscience ProbeMasterMix FAST Low ROX™ is shipped on wet ice but retain full activity at RT (+15°C to +25°C) for at least 3 days.

The Genaxxon bioscience ProbeMasterMix FAST Low ROX™ 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

ProbeMasterMix FAST Low ROX™ 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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