MANUAL

ProbeMasterMix FAST Low ROX™ (50nM)

qPCR Mastermix 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.

Component	Cat#	M3152.0200	M3152.1000	Colour code of cap
Premixed Mastermix for FAST real time PCR / qPCR without green fluorescence dye and with 50nM ROX™		2x 1mL for 200x 20µL reactions	10x 1mL for 1000x 20µL reactions	red

Description

The **Genaxxon ProbeMasterMix FAST – Low ROX™** is an advanced ready-to-use master mix optimized for probe-based FAST qPCR assays and instruments requiring low ROX™ as a reference dye (e.g., Agilent MX3000, MX3005P, MX4000, ABI 7500, ViiA™7). It contains a modified **HotStart Taq DNA polymerase** for high specificity and sensitivity, minimizing non-specific amplification and ensuring reliable results.

This mix includes **PCR buffer, dNTPs, $MgCl_2$, 50 nM ROX™** as a passive reference dye, and **performance-enhancing additives**. The hot-start DNA polymerase is **inactive at room temperature**, eliminating the need for cold setup conditions. The **advanced buffer system** ensures rapid reaction kinetics and efficient target amplification, even with **challenging templates** and **multiplex PCR applications**.

The 1 mL aliquot ensures **convenient and safe handling**.

Product specifications

Concentration:	2 time ready-to-use master mix
Additives:	50nM ROX™
Extension rate:	6-8 kb/min. at 72°C
5'-3' exonuclease activity:	Yes
Extra addition of A:	Yes
3'-5' exonuclease activity:	No
Standard Amplicon Size	100-400bp

Advantages

All-in-one master mix - for probe-based qPCR
High sensitivity
High efficiency and specificity
Wide dynamic range
High reproducibility
Hot start capacity - for room temperature setup (no pipetting on ice necessary)

Shipment: on wet ice but full activity at RT (+15°C to +25°C) for at least 3 days

Long-Term Storage: Stored at -20°C immediately upon receipt

Shelf-life: Stable until expiration date when stored and handled correctly

Unit definition

One unit is defined as the amount of enzyme which will convert 10 nmoles of dNTPs to an acid-insoluble form in 30 min at 72°C under the assay conditions (25 mM TAPS (tris-(hydroxymethyl)-methyl-amino-propanesulfonic acid, sodium salt) pH 9.3 (25°C); 50 mM KCl; 2 mM MgCl2; 1 mM b-mercaptoethanol; and activated calf thymus DNA as substrate.

Important notes before getting started

- This protocol is a guideline; optimize conditions as needed.
- 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 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.

MANUAL

PCR Protocol

Thaw primer solutions

Keep on ice after complete thawing and mix well before use.

Thaw ProbeMasterMix FAST at RT or on ice.

Keep on ice after thawing and mix well before use.

primer
template DNA
Nuclease-free H2O

Distribute 20 µL reaction mix into each tube.

Do not vortex!
Avoid bubbles - they interfere with the fluorescence detection!

Optional:

Prepare a primer mix of an appropriate concentration using sterile, 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 should not exceed 12.5µL per reaction.

It is very important to mix the ProbeMasterMix FAST well before use to avoid local differences in salt concentration.

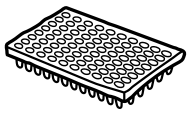
The Genaxxon bioscience ProbeMasterMix FAST is provided as a 2X concentrated mix (i.e. a 10µL volume of ProbeMasterMix FAST is required for qPCR reactions with a final volume of 20µL). For volumes smaller than 20µL, the 1:1 ratio of ProbeMasterMix FAST to diluted primer mix, template DNA and water should be maintained. A negative control (qPCR 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.

PCR protocol

Components	Quantities
ProbeMasterMix FAST	10 µL
primer 1:	1µL (200nM (100 - 400 nM recommended)
primer 2:	1µL (200nM (100 - 400 nM recommended)
Probe	1µL (400nM (200 - 600 nM recommended)
Template DNA*	0.1 - 10 ng per reaction
Nuclease-free water	up to 20µL

*For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume should not exceed 10% of the final PCR volume.

Choose 3 Step OR 2 Step PCR Protocol and FAST or Ultra-FAST Cycling conditions



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

A typical PCR cycling program is outlined on the right For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

*When using a thermal cycler with a heated lid, do not use mineral oil. Otherwise, overlay with app. 50µL mineral oil

FAST PCR conditions - 3 Step program

Step	Time	Temperature
Initial denaturation	**1-3 min**	**92 - 95 °C (important!)**
Denaturation	5 sec	92 - 95 °C
Annealing	5 sec	~60 °C*
Extension	5 - 10 sec	72 °C
Number of Cycles	25 - 40**	

*Approximately 5°C below Tm of primers
**For low copy number genes, it might be necessary to use cycle number of up to 45.

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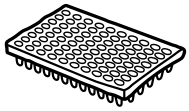

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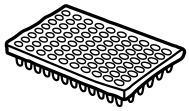
FAST PCR conditions - 2 Step program

Step	Time	Temperature
Initial denaturation	1-3 min	92 - 95 °C (important!)
Denaturation	5 sec	92 - 95 °C
Annealing/Extension	5 - 20 sec	~60 °C*
Number of Cycles	25 - 40**	

*Approximately 5°C below Tm of primers
**For low copy number genes, it might be necessary to use cycle number of up to 45.



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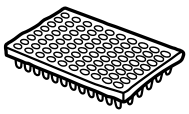
Ultra-FAST PCR conditions - 3 Step program

Step	Time	Temperature
Initial denaturation	1 min	92 - 95 °C (important!)
Denaturation	1 - 5 sec	92 - 95 °C
Annealing	1 - 5 sec	~60 °C*
Extension	1 sec	72 °C
Number of Cycles	25 - 40**	

*Approximately 5°C below Tm of primers
**For low copy number genes, it might be necessary to use cycle number of up to 45.



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

A typical PCR cycling program is outlined on the right For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

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Ultra-FAST PCR conditions - 2 Step program

Step	Time	Temperature
Initial denaturation	1 min	92 - 95 °C (important!)
Denaturation	1 sec	92 - 95 °C
Annealing/Extension	1 - 5 sec	~60 °C*
Number of Cycles	25 - 40**	

*Approximately 5°C below Tm of primers
**For low copy number genes, it might be necessary to use cycle number of up to 45.

Additional information

Applications

- **Probe-based FAST qPCR**
- **DNA Genotyping**
- **DNA SNP Analysis**
- **RNA and miRNA Expression**
- **Multiplexing (up to 4 colors)**
- **Transcript Variant Analysis**

Quality Control

Genaxxon bioscience ProbeMasterMix FAST 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

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).

Product Use Limitations

ProbeMasterMix FAST 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.

Further information

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