G:\products\productflyer\pcr\polymerasen\hotstart\3023_data.doc

Contact & Technical support

Tel.: +49 731 3608 123
Fax: +49 731 3608 962
e-mail: info@genaxxon.com

Genaxxon bioscience GmbH
Sölflinger Str. 70
D-89077 Ulm

www.genaxxon.com

GENAXXON bioscience

ProbeMasterMix (2X)

High ROX™

qPCR Mastermix without fluorescence dye
with 500nM passive reference dye

suited for example for following instruments:

ABi 5700, 7000, 7300, 7700, 7900, 7900HT, ABi Prism 7700
StepOne(TM) Plus, StepOne(TM), Eppendorf Realplex 4

Cat#: M3010

Version: 260116

Related Products

Cat #	Description
M3011	GreenMastermix Low ROX™ for Real-time PCR.
M3023	GreenMastermix without ROX™ for Real-time PCR.
M3052	GreenMastermix with High ROX™for Real-time PCR.
M3010	ProbeMastermix with High ROX™for Real-time PCR.
M3045	ProbeMastermix without ROX™for Real-time PCR.
M3307	SuperHotStart Polymerase for Real-time PCR / multiplex PCR.
M3006	HotStart Polymerase with antibody for Real-time PCR / multiplex PCR.
M3001	Taq-Polymerase with buffer S for high specifity PCR.
M3043	Taq-Polymerase with buffer E for high efficiency PCR.
M3014	Mastermix with Taq-Polymerase with buffer S for high specifity PCR.
M3029	Mastermix with Taq-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-Polymerase high efficiency proof-reading PCR (up to 7kb) and hotstart conditions.

“Your success is our aim”

For more information: www.genaxxon.com

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	

PCR Protocol Part

Protocol using Genaxxon ProbeMastermix (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.
- ProbeMastermix provides an optimized concentration of MgCl2 which will produce satisfactory results in most cases. However, if a higher Mg2+ concentration is required, the Genaxxon bioscience ProbeMastermix is shipped with additional 25mM MgCl2.
- 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 ProbeMastermix (2X)** at RT or on ice.
   Keep the solutions on ice after complete thawing. It is very important to mix the ProbeMastermix well before use to avoid local differences in salt concentration. The Genaxxon bioscience ProbeMastermix is provided as a 2X concentrated (i.e. a 12.5µL volume of ProbeMastermix is required for PCR reactions with a final volume of 25µL). For volumes smaller than 50µL, the 1:1 ratio of ProbeMastermix 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 ProbeMastermix.**
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.

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

Components	Quantities	Final concentration
ProbeMastermix	12.5µL	1X
primer forward (10µM)	0.5µL (0.25 - 2.5µL)*	0.1µM (0.05 - 0.5µM)**
primer reverse (10µM)	0.5µL (0.25 - 2.5µL)*	0.1µM (0.05 - 0.5µM)**
Probe (10µM) ** optional	x µL	0.05 - 1µM
Template DNA	x µL (variable volume)	genomic DNA: 20ng (1 - 100ng)*** plasmid DNA: 0.5ng (0.1 - 1ng)*** bacterial DNA: 5ng (1 - 10ng)***
sterile, bidestilled water	up to 25µL	

Keep all components on ice.
Spin down and mix all solutions carefully before use.
* Suggested starting conditions (Optimization of primer concentration is highly recommended)
** The necessary concentration of probe depends very much on the probe sequence and the kind of probe. Please test for optimum!
*** 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.**

Table 2: Final MgCl2 concentration in a **25µL reaction**

Additional MgCl2 conc. in reaction (mM)	0	0.5	1.0	1.5	2.0	2.5	3.0
Additional volume of 25mM MgCl2 per 25µL reaction (µL)	0	0.5	1.0	1.5	2.0	2.5	3.0

Note: The optimal Mg2+ concentration should be determined empirically but in most cases the optimized MgCl2 concentration, as provided with the ProbeMasterMix will produce satisfactory results.

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

Step	time	temperature	comments
Initial denaturation:	15 min.	95°C	
3-step cycling			
Denaturation:	15 - 30 sec.	95°C	
Annealing:	15 - 30 sec.	50 - 68°C	Approximately 5°C below Tm of primers.
Extension:	15 - 30 sec.	72°C	
Number of Cycles	25 - 35		
Final extension	2 min.	72°C	

Note: After amplification, samples can be stored at 4°C overnight, or -20°C for long term storage.

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

Step	time	temperature	comments
Initial denaturation:	15 min.	95°C	
3-step cycling			
Denaturation:	15 - 30 sec.	95°C	
Annealing:	30 - 60 sec.	60°C	Approximately 5°C below Tm of primers.
Number of Cycles	25 - 35		
Final extension	2 min.	72°C	

Note: After amplification, samples can be stored at 4°C overnight, or -20°C for long term storage.

This Genaxxon ProbeMastermix with High ROX™ is designed for example for the following instruments:

ABi 5700, 7000, 7300, 7700, 7900, 7900HT, ABi Prism 7700
StepOne(TM) Plus, StepOne(TM), Eppendorf Realplex 4

Hotline: +49 731 3608 123 or info@genaxxon.com