biomers.net
the biopolymer factory

ProbeMasterMix (2X)

No ROX™

qPCR Mastermix without fluorescence dye
without passive reference dye

suited for example for following instruments:

**Bio-Rad iCycler iQ, iQ5 and MyiQ™, CFX 96, CFX 480,
Chromo 4™ Real-Time Detector, DNA Engine Opticon™,
Mastercycler® ep realplex 2, Opticon® 2, Roche LightCycler® 96 and 480,
Rotor-Gene™ / Corbet, QuantStudio™, Smart Cycler®**

Art.no. MM10002

Related Products

Art.no.	Description
MM10001	ProbeMasterMix with High ROX™ for real time PCR / qPCR
MM10002	ProbeMasterMix without ROX™ for real time PCR / qPCR
MM10003	GreenMasterMix with High ROX™ for real time PCR / qPCR
MM10004	GreenMasterMix without ROX™ for real time PCR / qPCR

Manual Contents

Product	Art.no.	Package size
Premixed Mastermix for real time PCR / qPCR without green fluorescence dye, without ROX™: **Mix for 200 x 25µL reaction volume.**	MM10002	2 x 1.25mL (200 x 25 µL reactions)
Premixed Mastermix for real time PCR / qPCR without green fluorescence dye, without ROX™: **Mix for 1000 x 25µL reaction volume.**	MM10002	10 x 1.25mL (1000 x 25µL reactions)

Introduction

Quantitative PCR is an important tool for SNP and gene expression analysis. This ProbeMasterMix 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 biomers.net ProbeMasterMix (2X) is a ready-to-use PCR mixture that contains the chemically modified SuperHotStart *Taq* DNA Polymerase, 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 ProbeMasterMix without ROX™ contains special PCR buffer, $MgCl_2$, dNTPs and additives optimised for use in Real-Time PCR with specific oligonucleotide probes. 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 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 ProbeMasterMix is shipped with additional $MgCl_2$ solution. The small and convenient aliquot size of 1.25mL ensures and secures safe handling.

High Efficiency and Specificity

To examine the efficiency of the ProbeMasterMix 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 biomers.net ProbeMasterMix, respective the SuperHotStart Polymerase (figure 1).

This product is for research use only.

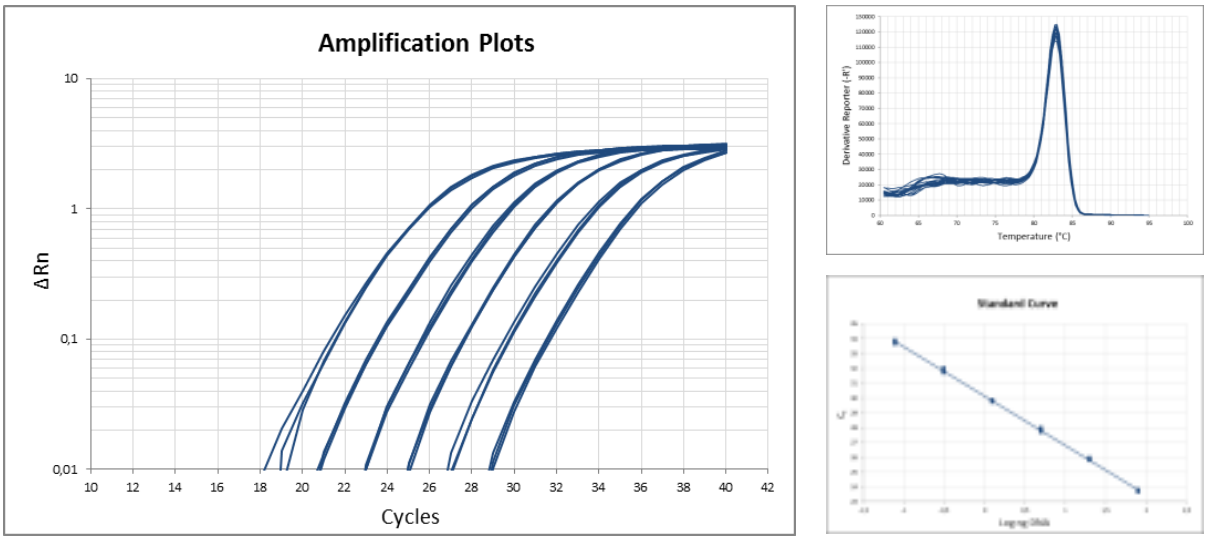

Figure 1: Amplification plot of a 4-fold dilution series for PAH target amplified from human gDNA. Starting amounts of 80ng gDNA, amplified in triplicates using ProbeMasterMix (2X) No ROX™.

Stability

Due to the stability of our ProbeMasterMix 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 ProbeMasterMix.

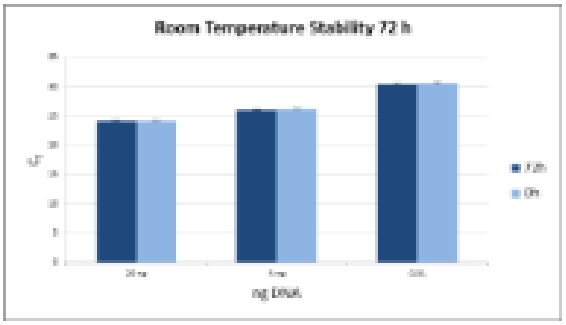

Figure 2: A PCR plate was pre-assembled for qPCR reaction and incubated in darkness at room temperature for 72 hours. The result shows high stability and inactivation of the SuperHotStart *Taq* DNA Polymerase before hot start.

Detection limit

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

Compatibility

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

Reproducibility

ProbeMasterMix (2X) No ROX™ produces reliable and reproducible results. A demonstration of 80 replicates, containing ProbeMasterMix (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).

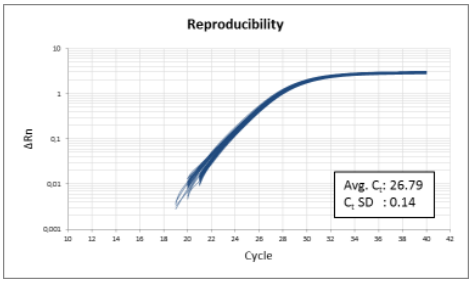

Figure 3: 80 replicates of the ProbeMasterMix (2X) No ROX™ and 20ng gDNA, show a standard deviation of only 0.14.

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:	Linearised 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 biomers.net ProbeMasterMix 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 optimised mastermix, without green dye
- High sensitivity
- High efficiency and high specificity
- Wide dynamic range
- High reproducibility
- Hot start capacity for room temperature setup

Supplied buffers/solutions

- *Magnesium stock solution*: 25mM $MgCl_2$

Stability

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

The biomers.net ProbeMasterMix, 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 biomers.net real time mastermixes can also be stored unopened at +2 to +8°C up to 10 months without loss of activity.

Product Use Limitations

ProbeMasterMix 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@biomers.net).

PCR Protocol Part

Protocol using biomers.net 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 optimised concentration of $MgCl_2$ which will produce satisfactory results in most cases. However, if a higher Mg^{2+} concentration is required, the biomers.net ProbeMasterMix is shipped with additional 25mM $MgCl_2$.
- 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 minimise 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 biomers.net 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 (Optimisation 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 optimised for each new target or primer pair.

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

Table 2: Final $MgCl_2$ concentration in a **25µL reaction**

Additional $MgCl_2$ conc. in reaction (mM)	0	0.5	1.0	1.5	2.0	2.5	3.0
Additional volume of 25mM $MgCl_2$ per 25µL reaction (µL)	0	0.5	1.0	1.5	2.0	2.5	3.0

Note: The optimal Mg^{2+} concentration should be determined empirically but in most cases the optimised $MgCl_2$ concentration, as provided with the ProbeMasterMix will produce satisfactory results.

Table 3: PCR conditions – Three step Program (**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. *	55°C – 65°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 +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 mastermixes!

Table 4: PCR conditions – Two step Program (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	15 min.	95°C	
2-step cycling			
Denaturation:	15 - 30 sec. *	95°C	
Annealing:	30 – 60 sec. *	55°C - 65°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 +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 mastermixes!

This biomers.net ProbeMasterMix without ROX™ is designed for example for the following instruments:

**Bio-Rad iCycler iQ, iQ5 and MyiQ™, CFX 96, CFX 480,
Chromo 4™ Real-Time Detector, DNA Engine Opticon™,
Mastercycler® ep realplex 2, Opticon® 2, Roche LightCycler® 96 and 480,
Rotor-Gene™ / Corbet, QuantStudio™, Smart Cycler®**

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%
Xylene cyanol	10000bp - 4000bp	750bp – 200bp
Cresol Red	2000bp – 1000bp	200bp – 125bp
Bromophenol blue	500bp – 400bp	150bp – 50bp
Orange G	<100bp	<20bp
Tartrazine	<20bp	<20bp

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Customer Service

You have questions or suggestions?
Contact us! We will be glad to assist you!

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