AnyGenes®

Efficient technologies for signaling pathways

Perfect Master Mix PROBE Kits (with ROX)

User Guide

For AnyGenes® products:

- ✓ Cat # PMP1-R50
- ✓ Cat # PMP1-R100
- ✓ Cat # PMP1-R200
- ✓ Cat # PMP1-R500

For research use only

Store at -20°C & keep away from light

2017

Summary

I. Product information

1) Introduction

The **qPCR Perfect Master Mix PROBE** is an optimised and convenient premix of the components (**except primers, probes and template**) necessary to perform real time polymerase chain reaction (qPCR).

It contains a thermo-stable Taq DNA Polymerase as well as buffer and $MgCl_2$ at concentrations optimised for the high performance of the enzyme, dNTPs required for amplification of DNA targets. In addition, the Master Mix contains **ROX** : be careful, the use of this passive reference dye is only compatible with some qPCR instruments (see compatibility in §5).

This ideal premix solution requires only the addition of your template (cDNA), primers and probes to perform your qPCR. The performance of AnyGenes® qPCR Perfect Master Mix PROBE has been carefully designed to provide you a high sensitivity and specificity. For details see www.anygenes.com.

✓ **Quality Control**

As part of our routine quality assurance program, all AnyGenes® products are monitored to ensure the highest levels of performance and reliability.

2) Intended use & licencing

For molecular biology research use only. This kit is not intended for diagnosis, prevention or therapeutic applications. AnyGenes® will be not responsible of the misuse of their products.

Purchase of AnyGenes® kits does not include or provide licence with respect to any patents owned by Hoffman-La Roche or others.

3) Kit contents

The Perfect Master Mix PROBE is supplied in 2X concentration. This Master Mix is optimised to use with probes and it contains:

- Optimised buffer components including $MgCl_2$
- Hot Start Taq DNA Polymerase
- dNTPs
- ROX

These Perfect Master Mix PROBE products are available in several formats :

Catalog Ref :	Contents	Contents
PMP1-R50	50 reactions	Perfect Master Mix PROBE (with ROX) : 1 x 0.5mL + PCR grade H2O : 1mL
PMP1-R100	100 reactions	Perfect Master Mix PROBE (with ROX) : 1 x 1mL + PCR grade H2O : 1mL
PMP1-R200	200 reactions	Perfect Master Mix PROBE (with ROX) : 2 x 1mL + PCR grade H2O : 1mL
PMP1-R500	500 reactions	Perfect Master Mix PROBE (with ROX) : 5 x 1mL + PCR grade H2O : 2 x 1mL

For more product information, please visit **www.anygenes.com** or contact us at **technical@anygenes.com**

4) Storage & stability

Upon receipt, store Perfect Master Mix PROBE kits at -20°C until their use. These storage conditions guarantee a long-term storage of AnyGenes® products for a minimum period of six months after their receipt. Moreover, in order to guarantee the stability of these products, avoid repeated freezing and thawing cycles. If small volumes of Perfect Master Mix PROBE are frequently required, we recommend to stock alicots at -20°C.

5) Additional reagents and equipment required

A) Reagents :

- Diluted template (cDNA)
- qPCR 96- or 384-well plate
- Ultra-pure & sterile « nuclease, *RNAse, DNAse free »* H_2O *(supplied with AnyGenes®* Perfect Master Mix PROBE *kit*)

Caution: Do not use DEPC H_2O !!!

B) Material :

- Real-time quantitative PCR instrument (Light Cycler® 480 (Roche®), ABI 7900®, ABI 7500® (Applied Biosystems® / Life Technologies®)...) *
- PCR plates centrifuge
- Vortex mixer and Mini-centrifuge
- “nuclease, RNase, DNase free” tips and tubes
- Pipettes for reaction mix preparation
- Disposable reagent reservoirs to dispense the reaction mix with multichannel pipettes

*Real-time qPCR instruments compatible with the use of **PMP kits (with ROX)** are listed below:

Company	Instruments	Perfect Master Mix PROBE Cat #
Applied Biosystems	Step One™ Real-Time System	PMP1-R
	Step One Plus™ Real-Time System	PMP1-R
	ABI 5700	PMP1-R
	ABI 7000	PMP1-R
	ABI 7300	PMP1-R
	ABI 7700	PMP1-R
	ABI 7900 HT (standard block)	PMP1-R
	ABI 7900 HT (FAST block)	PMP1-R
Eppendorf	Mastercycler™ ep realplex 2	PMP1-W / PMP1-R
	Mastercycler™ ep realplex 2S	PMP1-W / PMP1-R
	Mastercycler™ ep realplex 4	PMP1-W / PMP1-R
	Mastercycler™ ep realplex 4S	PMP1-W / PMP1-R

* LightCycler® Nano, 1, 2, 96 and 480 are trademarks of Roche. Step One™, Step One Plus™ Real-Time System, ABI 5700, ABI 7000, ABI 7300, ABI 7500, ABI 7700,7900HT, ViiA7™ system, QuantStudio™ Systems are trademarks of Applied Biosystems. iCycler™ iQ, iQ™5, MyiQ™, MyiQ2™, Chromo4™, Mini Opticon™, Opticon™, Opticon2™, CFX Connect™, CFX96, CFX384 are trademark of Bio-Rad. Mastercycler™ ep realplex is a trademark of Eppendorf. Mx3000P™, Mx3005P™, Mx4000™ and AriaMx™ are trademarks of Stratagene. Quantica and PrimeQ is a trademark of Techne.

*[2] **NB: WE INFORM YOU THAT PERFECT MASTER MIX PROBE DEVELOPPED BY ANYGENES® (STANDARD HOT-START ENZYMES) ARE NOT SUITABLE FOR USE IN FAST MODE ON APPROPRIATE QPCR INSTRUMENTS.**

For more product information, please visit www.anygenes.com or contact us at technical@anygenes.com

II. Protocol

1) Before you start...

To obtain reliable and reproducible results and avoid contamination and false-positive signals, it is important and necessary to follow Good Laboratory Practices.

Moreover, you don't need to add $MgCl_2$ in your PCR reaction mix. The concentration of $MgCl_2$ in our kits has already been adjusted to improve efficiency, specificity and repeatability.

2) Procedure

1) Thaw AnyGenes® Perfect Master Mix PROBE and your cDNA samples 20 minutes before use, in order that slowly reaches room temperature. You can also work with your samples on ice.

2) Prepare the work area (highly recommended under workstation) by carefully cleaning all material and areas with a suitable detergent and then decontaminating the workstation through exposure to UV.

3) Meanwhile, briefly centrifuge tubes and reagents and prepare the reaction mix in a 2 ml tube or directly in a disposable reagent reservoir according to the following table:

Reagents	**For 20 µl qPCR final reaction volume**	**For 10 µl qPCR final reaction volume**
	Volumes / reaction	**Volumes / reaction**
2X Perfect Master Mix PROBE	**10 µl**	**5 µl**
Primers (Forward & Reverse)	**... µl**	**... µl**
Probe	**... µl**	**... µl**
Ultra-pure H_20	***qsp 18 µl***	***qsp 9 µl***
Mix Volume	**18 µl per reaction**	**9 µl per reaction**
Diluted cDNA template	**+ 2 µl**	**+ 1 µl**
Total qPCR Reaction Volume	**20 µl per well**	**10 µl per well**

For more convenience and for high specificity and efficiency of your experiments, we have optimized our specific primers and probe design and composition of our kits so as to use together for best specific and reliable qPCR results.

Suggested composition for probe based detection :

- 10 pmols of primers Forward + Reverse, so a working concentration of 500 nM in a 20µl reaction
- 2 pmols of probe, so a working concentration of 100 nM in a 20µl reaction

4) Mix thoroughly with a pipette or briefly centrifuge the mix and tip out this mix in a disposable reagent reservoir.

5) According to your qPCR plate format, dispense 9 or 18 µl per well of the mix on the qPCR plate.

NB: Change tips to avoid cross contamination once it is necessary.

6) Add respectively 1 or 2 µl of cDNA (or H_2O for negative controls) on each well of your 96- or 384-well plate.

7) Cover the plate with a suitable optical sealing foil.

Caution: Do not prepare your PCR mix too early to ensure reliable and reproducible results. However, if your plate was prepared before the start of the qPCR run, keep the qPCR plate on ice or at 4°C in a refrigerator.

8) Centrifuge the plate 15-60 s at 1 000 g to remove any bubbles.

9) Meanwhile, prepare and check the run program under the following qPCR conditions (compatible with most qPCR instruments):

Phase	Number of cycles	Time	Temperature	Acquisition mode	Commentaries
Initial denaturation - HOT start Taq activation	**1**	**10 min**	**95°C**	**/**	**« Hot-start DNA Taq polymerase » activation**
Amplification	**40-45**	**10 s**	**95°C**	**/**	**Denaturation of cDNA brands**
		30 s	**60°C**	**quantification**	**Hybridation & elongation steps with fluorescence acquisition**

For further information, please contact technical support AnyGenes® via technical@anygenes.com

10) Place the qPCR plate in your qPCR instrument.

11) Start the qPCR run, following the manufacturer's recommendations and protocols.

III. Additional Informations

For further information, please contact technical support AnyGenes® via the following email address: **technical@anygenes.com**

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