AnyGenes®

Efficient technologies for signaling pathways

Perfect Master Mix SYBR® Green Kits

User Guide

For AnyGenes® products:

- ✓ Cat # PMSX-W2S
- ✓ Cat # PMSX-W4S
- ✓ Cat # PMSX-W8S
- ✓ Cat # PMSX-W12S
- ✓ Cat # PMSX-W24S

(* Cat # for all Perfect Master Mix SYBR® Green references compatible with SignArrays® system developed by AnyGenes®)

- ✓ Cat # PMS1-W50
- ✓ Cat # PMS1-W100
- ✓ Cat # PMS1-W200
- ✓ Cat # PMS1-W500

For research use only

Store at -20°C & keep away from light

2017

Summary

I. Product information

1) Introduction

The **qPCR Perfect Master Mix SYBR® Green** is an optimised and convenient premix of the components (**except primers 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, and SYBR® Green. For information, this kit contains no passive reference dye like ROX, nor fluorescein.

This ideal premix solution requires only the addition of your template (cDNA) and primers to perform your qPCR. The performance of AnyGenes® Perfect Master Mix SYBR® Green 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.

SYBR® Green is a registered trademark of Molecular Probes Inc.

Other brands or product names are trademarks of their respective holders.

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 SYBR® Green is supplied in 2X concentration. This Master Mix is optimised to use for SYBR® Green conditions and it contains:

- Optimised buffer components including $MgCl_2$ and SYBR® Green
- Hot Start Taq DNA Polymerase
- dNTPs

These Perfect Master Mix SYBR® Green products are available in several formats compatible with SignArrays® systems:

Catalog Ref :	Contents for SignArrays 96® system	Contents for SignArrays 384® system
PMSX-W2S	Perfect Master Mix SYBR® Green : 2 x 1mL + PCR grade H2O : 1mL	Perfect Master Mix SYBR® Green : 4 x 1mL + PCR grade H2O : 1mL
PMSX-W4S	Perfect Master Mix SYBR® Green : 4 x 1mL + PCR grade H2O : 1mL	Perfect Master Mix SYBR® Green : 8 x 1mL + PCR grade H2O : 1mL
PMSX-W8S	Perfect Master Mix SYBR® Green : 8 x 1mL + PCR grade H2O : 2 x 1mL	*
PMSX-W12S	Perfect Master Mix SYBR® Green : 12 x 1mL + PCR grade H2O : 2 x 1mL	*
PMSX-W24S	Perfect Master Mix SYBR® Green : 24 x 1mL + PCR grade H2O : 4 x 1mL	*
PMS1-W50	Perfect Master Mix SYBR® Green : 1 x 0.5mL + PCR grade H2O : 1mL	
PMS1-W100	Perfect Master Mix SYBR® Green : 1 x 1mL + PCR grade H2O : 1mL	
PMS1-W200	Perfect Master Mix SYBR® Green : 2 x 1mL + PCR grade H2O : 1mL	
PMS1-W500	Perfect Master Mix SYBR® Green : 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 SYBR® Green 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 SYBR Green® are frequently required, we recommend to stock alicots at -20°C.

5) Additional reagents and equipment required

A) Reagents :

- Diluted template (cDNA)
- qPCR plate or SignArrays® 96- or 384-well plates
- Ultra-pure & sterile « nuclease, *RNAse, DNAse free* » H_2O (*supplied with AnyGenes®* Perfect Master Mix SYBR® Green *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 **PMS kits (without ROX or fluorescein)** are listed below :

Company	Instruments	SignArrays 96® Cat #	SignArrays 384® Cat #	Perfect Master Mix SYBR® Green Cat #
Roche	LightCycler® Nano	*	*	PMSX-WXS
	LightCycler® 1 & 2	*	*	PMSX-WXS
	LightCycler® 96	XXXH1-RXS	*	PMSX-WXS
	LightCycler® 480	XXXH1-RXS	XXXH2-RXS	PMSX-WXS
Bio-Rad	Chromo4™	XXXH1-BXS	*	PMSX-WXS
	Mini Opticon™	*	*	PMSX-WXS
	Opticon™	XXXH1-BXS	*	PMSX-WXS
	Opticon2™	XXXH1-BXS	*	PMSX-WXS
	CFX Connect™	XXXH1-BXS	*	PMSX-WXS
	CFX96	XXXH1-BXS	*	PMSX-WXS
	CFX384	*	XXXH2-AXS	PMSX-WXS
Eppendorf	Mastercycler™ ep realplex 2	XXXH1-AXS	*	PMSX-WXS / PMSX-RXS
	Mastercycler™ ep realplex 2S	XXXH1-AXS	*	PMSX-WXS / PMSX-RXS
	Mastercycler™ ep realplex 4	XXXH1-AXS	*	PMSX-WXS / PMSX-RXS
	Mastercycler™ ep realplex 4S	XXXH1-AXS	*	PMSX-WXS / PMSX-RXS

* 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 SYBR GREEN® 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.

Caution: Perfect Master Mix SYBR® Green contains SYBR® Green, a DNA binding dye, which potentially have a carcinogen effect. Therefore, it is strongly essential to avoid inhalation and contact of this product with skin and mucous membranes.

2) Procedure

1) Thaw AnyGenes® Perfect Master Mix SYBR® Green 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:

- With standard qPCR plates :

Reagents	For 20 µl qPCR final reaction volume	For 10 µl qPCR final reaction volume
	Volumes / reaction	Volumes / reaction
2X Perfect Master Mix SYBR® Green	**10 µl**	**5 µl**
Primers (Forward & Reverse)	**... 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 primer based detection :

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

- For SignArray® system :

Reagents	For SignArrays® 96 system		For SignArrays® 384 system	
	Volumes / reaction	Recommended volumes for 1 SignArray® 96	Volumes / reaction	Recommended volumes for 1/4 SignArray® 384
2X Perfect Master Mix SYBR® Green	**10 µl**	**1 000 µl**	**5 µl**	**500 µl**
Ultra-pure H_20	**8 µl**	**800 µl**	**4 µl**	**400 µl**
Diluted cDNA template	**2 µl**	**200 µl**	**1 µl**	**100 µl**
Total Reaction Volume	**20 µl per well**	**2 000 µl**	**10 µl per well**	**1 000 µl**

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

NB: In qPCR SignArrays® 96 or 384 systems, primers are already lyophilized in the plates and you should not add primers in the reaction mix. For this application, please refer to the SignArray® system adapted protocol.

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.
   If you use the SignArray® system, dispense directly 10 or 20 µl per well of the reaction mix on the qPCR plate, as it already contains cDNA.

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.
   NB : Do not add cDNA template at this step if you use the SignArray® system !

7) Depending on the SignArray® system format, dispense 10 or 20 µl per well of the reaction mix on the qPCR plate.

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

8) 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.

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

10) 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
Melting curves	1	10 s	95°C	/	Melting curves
		30 s	65°C	/	
		0 s	95°C	continuous	

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

11) Place the SignArray® in your qPCR instrument.

12) 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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