AnyGenes®

Efficient technologies for signaling pathways

Validated Human Primer sets

Forward & Reverse Validated Primers mix sets - 50, 100, 200 or 500 qPCR reactions - 20X

Available in several species (*Homo sapiens*, *Mus musculus*, *Rattus norvegicus*,...)

For research use only

Store at -20°C & keep away from light

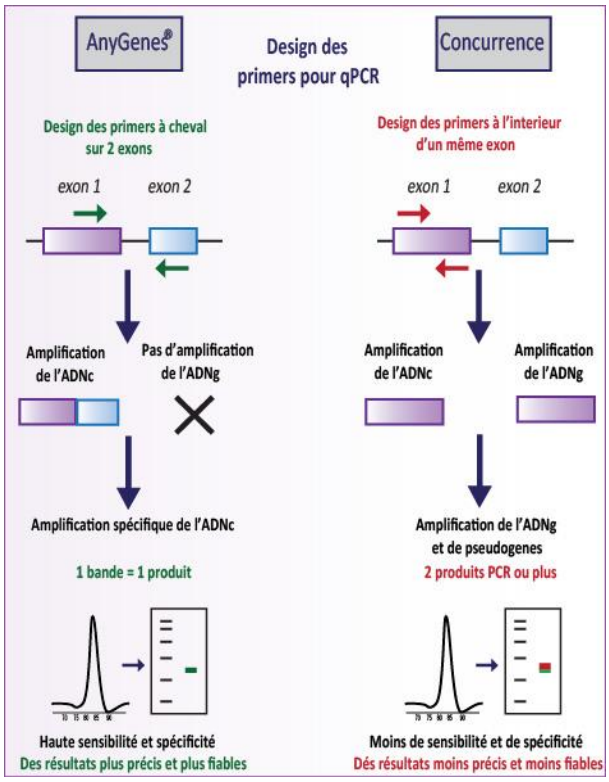
2017

SUMMARY

I. Product information

1) Introduction

The **Validated Primers sets** are primer pairs (Forward + Reverse) usable in **qPCR (or PCR) applications** designed preferentially on 2 different exons with very stringent criteria and **experimentally validated** thanks to our strict quality control process.



The performance of AnyGenes® Validated Primer sets has been carefully designed and optimized with our own Perfect Master Mix SYBR® Green kits 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

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.

3) Kit contents

This validated primer set contains according to our catalog reference :

Catalog Ref :	Contents	Number of reaction
HE-XXXX-50	One **HE-XXXXX-50** vial (50 reactions / 20X)	50 reactions
HE-XXXX-100	One **HE-XXXXX-100** vial (100 reactions / 20X)	100 reactions
HE-XXXX-200	One **HE-XXXXX-200** vial (200 reactions / 20X)	200 reactions
HE-XXXX-500	One **HE-XXXXX-500** vial (500 reactions / 20X)	500 reactions

***** **HE** catalog references for **Human Expression, HCN** for **Human gDNA CNV,** or **HP** for **Human gDNA promotor** and including **H** for ***Homo sapiens*** species, **M** for ***Mus musculus*** species, or **R** for ***Rattus norvegicus*** species
***** **XXXX for the gene name** (according to the HGNC approved-gene nomenclature)

According to your project, the design of our primers can be made to respond to several applications :

- Gene expression (design made in exonic regions) : cat # HE-...*
- Copy Number Variation (CNV) (design made in exonic and intronic gDNA regions : cat # HCN-...*
- Promoter screening (design made on promoter sequences) : cat # HP-...*

** example of cat # types for Homo sapiens species for each PCR or qPCR applications*

We recommend to use our Validated Primer sets with our Perfect Master Mix SYBR Green® kits, optimized together for best specific and reliable results. Moreover, make sure you have ordered the Perfect Master Mix SYBR Green® compatible with our qPCR instrument before starting the procedure.

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

4) Storage & stability

Upon receipt, store Validated Primer sets at -20°C until their use. These storage conditions guarantee a long-term storage of AnyGenes® products for a minimum period of 1 year after their receipt. Moreover, in order to guarantee the stability of these products, avoid repeated freezing and thawing cycles. If small volumes of Validated Primer sets are frequently required, we recommend to stock alicots at -20°C.

5) Additional reagents and equipment required

A) Reagents :

- Diluted template (cDNA)
- Perfect Master Mix SYBR Green® kits or equivalent (SYBR Green® based kits for qPCR applications)

- Ultra-pure & sterile « nuclease, *RNAse, DNAse free »* H_2O

Caution : Do not use DEPC H_2O !!!

B) Material :

- 96- or 384-well qPCR plates or equivalent
- 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

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.

2) Procedure

1) Thaw AnyGenes® Perfect Master Mix SYBR Green® or equivalent 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 **for each Validated Primer set** in a 2 ml tube according to the following table :

Reagents	Volumes / reaction	Volumes x n reactions
2X Perfect Master Mix SYBR Green®	**10 µl**	**n x 10 µl**
Ultra-pure H_20	**7 µl**	**n x 7 µl**
Validated Primer set 20X (Forward + Reverse, 10µM)	**1 µl**	**n x 1 µl**
Total Reaction Volume	**18 µl**	**n x 18 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.

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

5) Dispense **18 µl per well of the reaction mix** on the qPCR plate or equivalent. Then **add 2 µl of your diluted cDNA** per well, according to your handling plan.

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

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

Caution: Do not prepare your qPCR 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.

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

8) 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

9) Place the SignArray® in your qPCR instrument.

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

V. Additional Information

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

AnyGenes®
4 rue de la chine,
75970 Paris cedex 20
T: 00 33 (0)1 43 58 88 63
F: 00 33 (0)1 56 01 64 58

www.anygenes.com