

**PrimerDesign Ltd**

***Precision***<sup>TM</sup>

# **2X qPCR Mastermix**

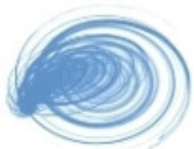
Instructions for use of PrimerDesign *Precision*<sup>TM</sup> Mastermix





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## Introduction

PrimerDesign Ltd *Precision*<sup>TM</sup> 2X qPCR Mastermix is optimized for use in qPCR. The Mastermix contains a thermo-stable TAQ Polymerase as well as buffer and MgCl<sub>2</sub> at concentrations optimised for the enzyme. In addition the Mastermix contains dNTPs required for amplification of a DNA target.

This complete solution requires only the addition of your cDNA, primers and probe to be PCR ready.

The performance of *Precision*<sup>TM</sup> 2X qPCR Mastermix is comparable to other leading brands. For details see [www.primerdesign.co.uk](http://www.primerdesign.co.uk)

### Guide to Hardware compatibility

Manufactures use varying methods to calibrate a real-time PCR reaction. For this reason the correct *precision*<sup>TM</sup> Mastermix formulation must be use for each platform.

Cat Number	Product Description	Compatible Hardware
Precision	<i>Precision</i> <sup>TM</sup> MasterMix	MJ Chromo4, Roche lightcycler 480 Platforms
Precision-LR	<i>Precision</i> <sup>TM</sup> MasterMix with LOW ROX	Applied Biosystems 7500 platform
Precision-R	<i>Precision</i> <sup>TM</sup> MasterMix with ROX	Applied Biosystems 7700, 7000, and 7900, 7300 platforms
Precision-iC	<i>Precision</i> <sup>TM</sup> MasterMix for the BioRad iCycler	BioRad iCycler. IQ4 and IQ5 platforms
Precision-MX	<i>Precision</i> <sup>TM</sup> MasterMix for Stratagene hardware	Stratagene MX platforms
Precision-CL	<i>Precision</i> <sup>TM</sup> MasterMix for capillary lightcyclers	Roche Capillary Lightcycler

### SYBRgreen based detection

The above kits are for probe based detection chemistry. If you are using primer only kits then the addition of SYBRgreen is required in the Mastermix. Mastermix with SYBRgreen can be supplied at no extra charge by adding "-SY" to catalogue number.



## Kit Contents

- **8 X 1.6ml aliquots of Precision qPCR Mastermix**  
Each tube contains 2x reaction buffer, 0.025 U/μl Taq Polymerase, 5 mM MgCl<sub>2</sub>, dNTP Mix (200μM each dNTP)

## Recommended Accompanying Products

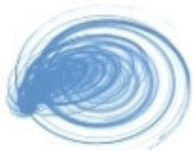
- **PrimerDesign custom designed real-time PCR primers**
- **PrimerDesign Precision™ Reverse Transcription kit** for production of cDNA template

## Reagents and Equipment to Be Supplied by User

- **Real-Time PCR Instrument**
- **Pipettors and Tips**
- **Vortex and centrifuge**

## Kit Storage

The PrimerDesign Precision™ 2X qPCR Mastermix kit should be stored at -20°C on arrival. Repeated freeze/thawing will not compromise the performance of the product. Under these conditions reagents are stable for six months from date of resuspension.



## Suitable Sample Material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration and DNA integrity. Always run at least one negative control with the samples. To prepare a negative-control, replace the test sample with RNase/DNase free water.

## Licensing Agreement and Limitations of Use

PCR is covered by several patents owned by Hoffman-Roche Inc and Hoffman-LaRoche, Ltd. Purchase of PrimerDesign kits does not include or provide licence with respect to any patents owned by Hoffman-La Roche or others. SYBR<sup>®</sup> green is a registered trademark of Molecular Probes Inc.

## PrimerDesign Ltd Satisfaction Guarantee

PrimerDesign takes pride in the quality of all our products. Should this product fail to perform satisfactorily when used according to the protocols in this manual, PrimerDesign will replace the item free of charge.

## Quality Control

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



## Bench-side Protocol

- **When using PrimerDesign gene detection kits.**

For each 20µl real-time PCR reaction add the following to each reaction tube

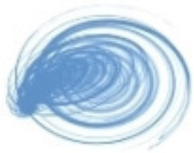
Components	1 Reaction
<i>Precision</i> <sup>TM</sup> 2X qPCR Mastermix	10 µl
Primer/Probe mix	1 µl
Template (25ng)	5 µl
RNAse/DNAse free water	4 µl
<b>Final volume</b>	<b>20 µl</b>

- **Suggested use with user supplied primers and probe.**

For each 20µl real-time PCR reaction add the following to each reaction tube

Components	1 Reaction
<i>Precision</i> <sup>TM</sup> 2X qPCR Mastermix	10 µl
Primers (6pmols Forward and Reverse)	x µl
Probe (3pmols)	x µl
Template (25ng)	x µl
RNAse/DNAse free water (up to Final volume)	x µl
<b>Final volume</b>	<b>20 µl</b>

\*6pmols of primer gives a working concentration of 300nM in a 20µl reaction



# Amplification Protocols

- For use with **PrimerDesign PerfectProbe™** gene detection kits

	Step	Time	Temp
	Enzyme Activation - HotStart	10 min	95°C
Cycling x50	Denaturation	15s	95°C
	<b>DATA COLLECTION*</b>	30s**	50°C
	Extension	15s	72°C

\*Fluorogenic data should be collect during this step through the FAM channel.

\*\* For some real-time PCR platforms 15 seconds is sufficient to acquire data through 2 channels. This step may be shortened accordingly. A 30s step is recommended for the Bio-Rad iQcycler.

- For use with **Taqman®** gene detection kits

	Step	Time	Temp
	Enzyme Activation - HotStart	10 min	95°C
Cycling x50	Denaturation	15s	95°C
	<b>DATA COLLECTION*</b>	60s	60°C

\*Fluorogenic data should be collect during this step through the FAM channel.

- For use with **SYBRgreen®** detection kits

	Step	Time	Temp
	Enzyme activation - HotStart	10min	95°C
Cycling x50	Denaturation	15s	95°C
	<b>DATA COLLECTION*</b>	60s	60°C
	Melt Curve**		

\*Fluorogenic data should be collect during this step through the SYBRgreen® channel.

\*\*A post PCR run melt curve can be used to prove the specificity of the primers. See the manufactures instructions for your hardware platform