

Precision[®]PLUS OneStep RT-qPCR Master Mix

Instructions for use of Primerdesign
Precision[®]PLUS OneStep Master Mix for real-time RT-qPCR

PRIMER
DESIGN

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Introduction

PrecisionPLUS OneStep RT-qPCR Master Mix is an optimised complete system for use in one step real-time PCR. Removal of a separate reverse transcription step reduces handling errors and greatly reduces the time taken to obtain results.

PrecisionPLUS OneStep RT-qPCR Master Mix contains a unique thermostable MMLV (Moloney murine leukemia virus) enzyme which has an optimal operating temperature of 55°C. It also has a higher affinity for primer template duplexes which allows very rapid processing during the RT step. This enables reverse transcription to be completed in under 10 minutes. The Master Mix also contains Taq Polymerase as well as buffer and MgCl₂ at concentrations optimised for the amplification step. Only the RNA template and primer and probe mix are required to complete the experimental set up for a perfect single tube reaction.

Guide to Hardware compatibility

Manufacturers use varying methods to calibrate a real-time PCR reaction. For this reason, the correct PrecisionPLUS OneStep Master Mix formulation must be used for each platform.

Cat Number	Product Description	Compatible Hardware
OSPLUS	Precision®PLUS OneStep RT-qPCR Master Mix	MJ Opticon and Chromo4 Roche lightcycler 480 and LC96 Biorad CFX Rotorgene, Eppendorf Mastercycler Fluidigm BioMark Cepheid SmartCycler Illumina Eco PCRMax Eco Thermo PikoReal PrimerPro 48 Analytik Jena qTower Series
OSPLUS-LR	Precision®PLUS OneStep RT-qPCR Master Mix with LOW ROX	Applied Biosystems 7500 ViiA7 Life Technologies QuantStudio
OSPLUS-R	Precision®PLUS OneStep RT-qPCR Master Mix with ROX	Applied Biosystems 7000 Applied Biosystems 7300 Applied Biosystems 7700 Applied Biosystems 7900 Applied Biosystems StepOne Applied Biosystems StepOnePLUS GeneAmp 5700 platforms
OSPLUS-IC	Precision®PLUS OneStep RT-qPCR Master Mix for the BioRad iCycler	BioRad iCycler IQ4 and IQ5
OSPLUS-MX	Precision®PLUS OneStep RT-qPCR Master Mix for Stratagene hardware	Stratagene MX platforms
OSPLUS-CL	Precision®PLUS OneStep RT-qPCR Master Mix for capillary lightcyclers	Roche Capillary Lightcycler 1.5 and 2.0.

If your real-time PCR machine is not listed. Please contact us via our website: www.primerdesign.co.uk

Pack sizes

The following pack sizes are available:

Cat Number	Pack size	Composition
OSPLUS-1ML	1ml	1 X 1ml aliquot
OSPLUS-2ML	2ml	2 X 1ml aliquots
OSPLUS-5ML	5ml	5 X 1ml aliquots
OSPLUS-10ML	10ml	10 X 1ml aliquots
OSPLUS-20ML	20ml	20 X 1ml aliquots

Just add the required pack size to the catalogue number

SYBR®green based detection

If SYBR®green is required in the mix then add ‘-SY’ to the catalogue number e.g. OSPLUS-R-SY

Inert blue loading dye

If inert blue loading dye is required, then choose the relevant catalogue number below and add this to your order when purchasing PrecisionPLUS OneStep Master Mix. You will be supplied with a separate vial of blue dye.

To use the inert blue loading dye, simply add 4.7µl to each 1ml vial of PrecisionPLUS OneStep Master Mix. Mix the blue dye into the Master Mix by inverting the tube.

Cat Number	Composition
BLUEDYE-1ML	Blue dye required to convert 1ml Master Mix
BLUEDYE-2ML	Blue dye required to convert 2ml Master Mix
BLUEDYE-5ML	Blue dye required to convert 5ml Master Mix
BLUEDYE-10ML	Blue dye required to convert 10ml Master Mix
BLUEDYE-20ML	Blue dye required to convert 20ml Master Mix

Kit contents

- PrecisionPLUS OneStep RT-qPCR Master Mix aliquots

Recommended accompanying products

- Primerdesign custom designed real-time PCR primers
- genesig pathogen detection kits
- Primerdesign real-time PCR internal control
- Primerdesign BrightWhite real-time PCR plasticware

Reagents and equipment to be supplied by user

- Real-Time PCR Instrument
- Pipettes and Tips
- Vortex and centrifuge

Kit storage

The Primerdesign PrecisionPLUS OneStep RT-qPCR Master Mix kit should be stored at -20°C on arrival. Repeated freeze/thawing should be avoided as it may compromise the performance of the product. Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of RNA sample material can be used (e.g. Viral RNA, cell culture derived RNA, Biopsy derived RNA etc). Please ensure the samples are suitable in terms of purity, concentration and RNA 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[®]green is a registered trademark of Molecular Probes Inc. PrecisionPLUS containing GoTaq[®] Hot Start Polymerase manufactured by Promega Corporation for distribution by Primerdesign Ltd. Licensed to Promega under U.S. Patent Nos. 5,338,671 and 5,587,287 and their corresponding foreign patents.

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 ISO9001 and ISO13485 quality assurance systems, all Primerdesign products are monitored to ensure the highest levels of performance and reliability.

Bench-side protocol

For optimum performance and sensitivity all pipetting steps and experimental **plate set up should be performed on ice**. After the plate is poured proceed immediately to the one step amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artefacts that reduce the sensitivity of detection.

When using Primerdesign kits:

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

Components	1 Reaction
PrecisionPLUS OneStep RT-qPCR Master Mix	10 µl
Primer/Probe mix	1 µl
Template RNA (25ng)	x µl
RNase/DNase free water (up to Final volume)	x µl
Final volume	20 µl

Suggested use with user supplied primers and probe:

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

Components	1 Reaction
PrecisionPLUS OneStep RT-qPCR Master Mix	10 µl
Primers* (6pmols forward and reverse)	x µl
Probe (3pmols)	x µl
Template RNA (25ng)	x µl
RNase/DNase free water	x µl
Final volume	20 µl

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

Amplification protocols

Precision[®]PLUS OneStep RT-qPCR Master Mix

For use with standard cycling protocols with double-dye gene detection kits

	Step	Time	Temp
	Reverse Transcription	10 min	55°C
	Enzyme Activation	2 min	95°C
Cycling x40**	Denaturation	10 sec	95°C
	DATA COLLECTION*	60 sec	60°C

*Fluorogenic data should be collected during this step through the appropriate channel.

** For low copy number targets, giving late detection, a further 10 cycles may be needed to generate the complete amplification plot

For use with SYBR[®]green detection chemistry

	Step	Time	Temp
	Reverse Transcription	10 min	55°C
	Enzyme activation	2 min	95°C
Cycling x40***	Denaturation	10 sec	95°C
	DATA COLLECTION*	60 sec	60°C
	Melt Curve**		

*Fluorogenic data should be collected during this step through the SYBR[®]green 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

*** For low copy number targets, giving late detection, a further 10 cycles may be needed to generate the complete amplification plot.