Precision®FAST qPCR Master Mix

Instructions for use of Primerdesign
Precision®FAST Master Mix for real-time PCR

PRIMER DESIGN

Contents

Introduction

Precision®FAST Master Mix

Primerdesign PrecisionFAST is designed for rapid cycling protocols that can dramatically shorten run times. The Taq Polymerase has been mutated at the active site and has higher affinity for DNA and faster processing. The buffer has been designed for optimum sensitivity and also to reduce primer dimers which are a common artefact of fast processing enzymes.

Guide to Hardware compatibility

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

Cat Number	Product Description	Compatible Hardware
PFAST	Precision®FAST 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 PrimePro 48 Analytik Jena qTower Series
PFAST-LR	Precision®FAST qPCR Master Mix with LOW ROX	Applied Biosystems 7500 ViiA7 Life Technologies QuantStudio
PFAST-R	Precision®FAST 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
PFAST-iC	Precision®FAST qPCR Master Mix for the BioRad iCycler	BioRad iCycler IQ4 and IQ5
PFAST-MX	Precision®FAST qPCR Master Mix for the Stratagene	Stratagene MX platforms
PFAST-CL	Precision®FAST qPCR Master Mix for 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
PFAST-1ML	1ml	1 X 1ml aliquot
PFAST-2ML	2ml	2 X 1ml aliquots
PFAST-5ML	5ml	5 X 1ml aliquots
PFAST-10ML	10ml	10 X 1ml aliquots
PFAST-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. PFAST-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 PrecisionFAST 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 PrecisionFAST 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

- **PrecisionFAST Master Mix aliquots**

Recommended accompanying products

- **Primerdesign custom designed real-time PCR primer or primer/probe mixes**
- **Primerdesign Precision nanoscript2 Reverse Transcription kit for production of cDNA template**
- **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**
- **Pipettors and Tips**
- **Vortex and centrifuge**

Kit storage

The Primerdesign PrecisionFAST qPCR Master Mix kit should be stored at -20ºC on arrival. Repeated freeze/thawing should be kept to a minimum to maximise performance of this product. Primerdesign does not recommend using the kit after the expiry date stated on the pack.

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

Bench-side protocol

When using Primerdesign kits:

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

Components	1 Reaction
PrecisionFAST qPCR Master Mix	10 µl
Primer/Probe mix	1 µl
Template (25ng)	5 µl
RNase/DNase free water	4 µl
Final volume	**20 µl**

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
PrecisionFAST qPCR Master Mix	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
Final volume	**20 µl**

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

Amplification protocols

Precision®FAST Master Mix

For use with double-dye gene detection kits

	Step	Time	Temp
	Enzyme Activation – Hot Start	2 min	95ºC
Cycling x40**	Denaturation	5 sec	95ºC
	DATA COLLECTION*	20 sec	60ºC

*Fluorogenic data should be collected during this step through the FAM 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 gene detection kits

	Step	Time	Temp
	Enzyme activation – Hot Start	2 min	95ºC
Cycling x40***	Denaturation	5 sec	95ºC
	DATA COLLECTION*	20 sec	60ºC
	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 manufacturer's 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