



TETRA™ ONESTEP 2X qRT-PCR MASTERMIX

CAT NO. YS-qP-TOSMM-5/10/20

5ml / 10ml / 20ml

VERSION 2.0

For Research Use Only



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INTENDED USE

YouSeq Tetra™ OneStep MasterMix is a convenient 2X mix designed for real-time PCR applications. The MasterMix formulation has been specifically engineered for highly processivity, powerful multiplexing capability and to be highly resistant to inhibitors.

The high accuracy and enhanced 5'-3' exonuclease activity of our Polymerase result in very efficient probe cleave activity for superior probe-based assay performance. The enzyme is ideally suited to applications where sensitivity and speed to result is key.

YouSeq Tetra™ OneStep MasterMix uses an advanced buffer system including dNTPs, Mg and enhancers, enabling efficient PCR of a wide range of target types from all sort of sequence types including small GC-rich genomes with minimal or no optimisation required.

KIT CONTENTS

Standard Packs:

	5 ml Pack Size	10 ml Pack Size	20 ml Pack Size
Tetra™ OneStep 2X qRT-PCR MasterMix	5 x 1 ml	10 x 1 ml	20 x 1 ml

Low ROX:

	5 ml Pack Size	10 ml Pack Size	20 ml Pack Size
Tetra™ OneStep 2X qRT-PCR MasterMix	5 x 1 ml	10 x 1 ml	20 x 1 ml
ROX Passive Reference	10 µl	10 µl	10 µl
DNase/RNase Free Water	1.5 ml	1.5 ml	1.5 ml

Mid ROX:

	5 ml Pack Size	10 ml Pack Size	20 ml Pack Size
Tetra™ OneStep 2X qRT-PCR MasterMix	5 x 1 ml	10 x 1 ml	20 x 1 ml
ROX Passive Reference	10 µl	10 µl	2 x 10 µl
DNase/RNase Free Water	1.5 ml	1.5 ml	1.5 ml

High ROX:

	5 ml Pack Size	10 ml Pack Size	20 ml Pack Size
Tetra™ OneStep 2X qRT-PCR MasterMix	5 x 1 ml	10 x 1 ml	20 x 1 ml
ROX Passive Reference	3 x 10 µl	5 x 10 µl	10 x 10 µl
DNase/RNase Free Water	1.5 ml	1.5 ml	1.5 ml

ROX (PLATFORM DEPENDENT)

ROX is required for platforms that use ROX as a passive reference. The table below outlines the hardware platforms that require the addition of ROX.

If ROX is required, dilute the ROX supplied according to the table below, then add 5µl to each of the Tetra™ MasterMix tubes.

	Instruments	Step 1: Volume of water to add to each ROX tube	Step 2: Add to MasterMix tube
High ROX Instruments	Applied Biosystems 7700, 7000, 7900, 7300, StepOne, StepOne Plus, and Roche capillary Lightcylers 2.0	No Dilution Required	5 µl
Mid ROX Instruments	Stratagene MX	75 µl	5 µl
Low ROX Instruments	Applied Biosystems 7500 Platform, ViiA7 platforms, Quantstudio	130 µl	5 µl
All Other Machines		Not Required	Not Required

SUGGESTED USE CONDITIONS AND OPTIMISATIONS

Reverse Transcription (RNA work only)

Reverse Transcription (for qRT-PCR work) is performed at 55°C to drive fast efficient conversion of RNA to cDNA with minimal template structure.

Denaturation

A denaturation step at 95°C is recommended. However, when using a template with high GC content, this may be increased to 98-100°C to improve results.

Annealing

The recommended annealing temperature is between 60 and 70 °C. The annealing temperature may be optimised by performing a temperature gradient starting at 60°C then increasing in 2°C increment to remove non-specific product.

Template Concentration

The recommended amount of template is 1-50 ng RNA per reaction, although the mix is extremely sensitive and capable of quantitation at pico-gram levels.

qPCR BENCH SIDE PROTOCOL

Clean and decontaminate all work surfaces, pipettes, and other equipment prior to use to remove potentially contaminating nucleic acids.

REACTION SET UP

Combine the following reagents to create a final test reaction:

Component	Volume
Tetra™ OneStep 2X qRT-PCR MasterMix	10 µl
Primer/probe mix	1 µl
Extracted Sample RNA	2-9 µl
DNase/RNase Free Water	x µl
Final Volume	20 µl

qPCR AMPLIFICATION PROTOCOL

Run the following PCR protocol:

	Temperature	Time
	55°C	10 minutes
Hot Start	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds

PRODUCT SPECIFICATIONS

Storing your kit

On arrival the kit should be stored between -30°C and -15°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can go through 10 freeze/thaw cycles with no loss of activity. We recommend storing the MasterMix in small aliquots if you plan to utilise it over more than 10 occasions.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of YouSeq Tetra™ OneStep 2X qRT-PCR MasterMix is tested against predetermined specifications to ensure consistent product quality.

Technical Assistance

For customer support, please contact:

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