YOUSEQ

TETRA™ 2X qPCR MASTERMIX

qPCR HANDBOOK

For Research Use Only. Not intended for diagnostic use.

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

5 mL / 10 mL / 20mL

VERSION 4.0

YOUSEQ

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YOUSEQ

INTENDED USE

YouSeq Tetra™ MasterMix is a 2X ready-to-use mix, enabling superior assay performance across various sample types. It is intended for use by trained professionals in a suitable molecular biology laboratory.

CONTENTS

Standard Packs:

Component	Cap Colour	5 mL Pack Size	10 mL Pack Size	20 mL Pack Size
Tetra™ 2X qPCR MasterMix		5 x 1 mL	10 x 1 mL	20 x 1 mL

Low ROX:

Component	Cap Colour	5 mL Pack Size	10 mL Pack Size	20 mL Pack Size
Tetra™ 2X qPCR MasterMix		5 x 1 mL	10 x 1 mL	20 x 1 mL
ROX Passive Reference Dye		1 x 10 µL	1 x 10 µL	1 x 10 µL
DNase/RNase Free Water		1 x 1.5 mL	1 x 1.5 mL	1 x 1.5 mL

High ROX:

Component	Cap Colour	5 mL Pack Size	10 mL Pack Size	20 mL Pack Size
Tetra™ 2X qPCR MasterMix		5 x 1 mL	10 x 1 mL	20 x 1 mL
ROX Passive Reference Dye		3 x 10 µL	5 x 10 µL	10 x 10 µL
DNase/RNase Free Water		1 x 1.5 mL	1 x 1.5 mL	1 x 1.5 mL

RECOMMENDED ADDITIONAL REAGENTS & MATERIALS

qPCR primer/probe mixes (Recommended for use with YouSeq extensive qPCR design catalogue: www.youseq.com).

Nucleic acid template.

General laboratory equipment (pipettes, pipette tips, (micro)centrifuge tubes, compatible strip tubes/plates, plate seals etc.)

qPCR instrument.

BEST PRACTICE

Decontamination:

Before beginning laboratory work, thoroughly decontaminate any work surfaces & pipettes being used, to eliminate potential contamination.

General use & set-up:

All components should be fully defrosted with contents at the bottom of the tube before opening. To ensure contents are at the bottom, centrifuge or gently tap the tube. After use, reagents should be returned to the freezer.

After resuspending or combining reagents, the tube should be pulse vortexed to ensure it is mixed well.

It is advised to set up the plate and reaction mix on ice to minimise artefact formation, which may reduce sensitivity.

When preparing the qPCR reaction mix, it is recommended to incorporate an overage when calculating the total number of reactions to compensate for potential volume losses incurred during pipetting.

Set-up environments:

It is best practice to set up qPCR tubes/plates in two different environments - a clean (no template) lab and PCR (template) lab.

BENCH SIDE PROTOCOL

ROX (INSTRUMENT DEPENDENT)

ROX can be required for instruments that use ROX as a passive reference, however, if using a qPCR primer/probe mix labelled with a ROX fluorophore, the passive reference dye should not be used. The table below outlines the qPCR instruments that require the addition of ROX to the MasterMix.

1. If ROX is required:
    a. Dilute the ROX supplied according to the table below based on the intended qPCR instrument to be used.
    b. Directly transfer 5 µL of prepared ROX to the Tetra™ MasterMix.

Level of ROX	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	No Dilution Required	5 µL
Low ROX Instruments	Applied Biosystems 7500 & 7500 FAST, ViiA7, Quantstudio, Stratagene MX	130 µL	5 µL
ROX Not Required:	All Other Instruments	Not Required	Not Required

qPCR REACTION SET-UP

Tetra™ qPCR MasterMix is supplied at 2X concentration. When setting up the qPCR reaction mix, the MasterMix is required to be half the total reaction volume.

Example: 10 µL of Tetra™ 2X qPCR MasterMix in a 20 µL total reaction volume.

qPCR AMPLIFICATION PROTOCOL

1. Load the tubes/plate onto the qPCR instrument and set up the recommended qPCR protocol below.

Please note: If using a qPCR instrument that uses ROX as a passive reference and a primer/probe mix containing a ROX labelled probe, ensure the passive reference is turned off or set to "none" before starting the run.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	3 minutes	-
Denaturation	95°C	15 seconds	x 45
Annealing/Extension	60°C 📷	60 seconds	

📷 Collect fluorogenic data through the required channels during this step.

2. Start the run.

The recommended qPCR protocol above can be adjusted following the details below to optimise the reaction for the intended application.

Uracil-DNA Glycosylase (UNG/UDG)

The Tetra™ 2X qPCR MasterMix contains dUTP's and Uracil-DNA Glycosylase (UNG/UDG). The enzyme will degrade DNA templates containing Uracil bases, in order to prevent carry-over contamination during PCR setup. However, it is designed to denature completely at 55°C and hence will not impact the PCR amplification.

Denaturation

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

Annealing/Extension

The recommended annealing temperature is 60°C. The annealing temperature may be optimised by performing a temperature gradient within the annealing step between 60°C and 70°C to remove non-specific product.

PRODUCT SPECIFICATIONS

Storage

Store at -20°C from arrival. The products shelf life is outlined as an expiry date on the pouch label.

To reduce excessive freeze-thaws (<10), the MasterMix can be stored in small aliquots if required.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use.

Quality Control

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

Technical Assistance

For customer support, please contact:

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