



YOUSEQ

HIGH-FIDELITY 2X MASTERMIX USER GUIDE

CAT NO. YS-NGS-HiFi-5/10/100

5ml, 10ml or 100ml

VERSION 2.0

For Research Use Only



YOUSEQ

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INTENDED USE

YouSeq High-Fidelity 2X MasterMix is a high-fidelity 2X mix designed for PCR applications where greater sequence accuracy is required, together with improved PCR success rates of long and challenging templates.

YouSeq High-Fidelity MasterMix contains an engineered Polymerase derived from Pfu DNA Polymerase with several proprietary mutations. The enzyme has improved DNA binding and processivity, amplifies longer and more difficult targets and produces higher yields. These characteristics result in shorter extension times (10-30s/kb), fidelity approximately 100 times higher than Taq DNA polymerase and ability to amplify genomic template in excess of 17.5kb.

Polymerase selection and the use of an advanced buffer system enables high-fidelity PCR of a wide range of targets and fragment sizes with minimal or no optimisation required. YouSeq High-Fidelity MasterMix has been specifically developed for fast and versatile high-fidelity PCR, ideally suited to applications where greater accuracy is required such as cloning, site-directed mutagenesis and sequencing. PCR products generated with this range of products are blunt ended.

Storage Conditions

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.

KIT CONTENTS

	5ml Pack Size	10ml Pack Size	100ml Pack Size
YouSeq High-Fidelity 2X MasterMix	5 x 1.1 ml	10 x 1.1 ml	100 x 1.1 ml

SUGGESTED USE CONDITIONS AND OPTIMISATIONS

Primers T_m

Primers with a predicted melting temperature of approx. 60°C are recommended

Primer Concentration

Primers should be used between 0.2µM and 0.6µM

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

Extension

Optimal extension is achieved at 72°C. It is dependent on amplicon length and template complexity. 30 seconds per kilobase (kb) is recommended.

Fast cycling

If using faster extension times, care must be taken to prevent loading too much template DNA. If non-specific bands are visible after amplification, the amount of template DNA should be decreased.

Template Concentration

The recommended amount of template is 5-100 ng genomic DNA per reaction

HIGH-FIDELITY 2X MASTERMIX

Combine the following reagents to set up a final test reaction:

Component	Volume
YouSeq HiFi MasterMix	12.5 µl
Primer Pool (e.g. 5µM)	2 µl
Template DNA	Volume equivalent to 5-100ng
DNase/RNase free water	x µl
Final Volume	25 µl

Run the test reaction on the following cycling conditions:

	Temperature	Time
	95°C	1 - 3 minutes
20-50* cycles	95°C	10-30 seconds
	60-72°C	1 - 8 mins**

*Cycle number dependant on application

**Choose longer annealing/extension time for larger multiplexes