

# User Guide



YOUSEQ

## High Fidelity 2x MasterMix

Version 1.2

### Regulatory status

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

# Product description

## Introduction

YouSeq High Fidelity MasterMix is a convenient 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 and highly processive Polymerase, developed for fast and versatile high-fidelity PCR. The enzyme is derived from Pfu DNA polymerase for its 3'-5' exonuclease (proofreading) activity.

Several proprietary mutations significantly improve DNA binding and processivity, resulting in shorter extension times (10-30s/kb), higher yields and the ability to amplify longer and more difficult targets, including eukaryotic genomic templates in excess of 17.5kb.

The high accuracy and enhanced 3'-5' exonuclease activity of our Polymerase result in fidelity that is approximately 100 times higher than Taq DNA polymerase. The enzyme is 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.

YouSeq High Fidelity MasterMix uses an advanced buffer system including dNTPs, Mg and enhancers, enabling high fidelity PCR of a wide range of targets and fragment sizes with minimal or no optimisation required.

## YouSeq Recommendations

### Primers T<sub>m</sub>

Predicted melting temperature of approx. 60°C

### Primer Concentration

Between 0.2µM and 0.6µM.

### Denaturation

Should be 95°C, however, presence of high GC template, increase it to 98-100°C to improve results

### Annealing

Recommend performing temperature gradient to determine optimal annealing temperature. We'd recommend 60°C annealing temperature then increase in 2°C increment to remove non-specific product

### Extension

Optimal Extension is achieved at 72°C. 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

We recommend using 5-100 ng genomic DNA

## Reaction Set-Up

Component	Volume
YouSeq NGS MasterMix	12.5 µL
Primer Pool (e.g. 5 µM)	2 µL
Template DNA	5-100ng
DNase/RNase free water	x µL
<b>FINAL VOLUME</b>	<b>25 µL</b>

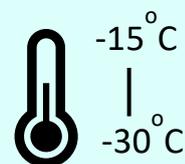
## Cycling Conditions

	Temp	Time
	95°C	1-3 mins
<b>20-40 cycles</b>	95°C	10-30 secs
	60°C-70°C	60 secs*

\*Choose longer annealing time for larger amplicons

## Storage Conditions

On arrival the kit should be stored between -30°C and -15°C.



- Avoid prolonged light exposure
- If stored correctly, retain full activity for at least 12 months

## Freeze Thaws (F.T) ❄️

- The kit can go through 10 freeze/thaw cycles with no loss of activity.
- If plan >10 F/T, aliquot the MasterMix into smaller volumes