

User Manual

Version 3.0

Product name: I-5™ 2X High-Fidelity Master Mix

Cat #: I5HM-100, I5HM-200, I5HM-5000, I5HM-25k, I5HM-OEM

Description:

I-5™ High-Fidelity DNA Polymerase is an ultra-high fidelity and high processivity enzyme. It produces the most accurate copies of DNA, and performs at an ultra-high rate. I-5™ is perfect for applications like cloning, in vitro amplifying materials for protein expression, SNP analysis by sequencing, and high-specificity PCR.

Convenient and Usage:

I-5™ 2X High-Fidelity Master Mix has a buffer system that is designed to offer maximum performance of the I-5™ High-Fidelity DNA Polymerase. It offers the highest fidelity and incredibly robust performance. The convenient master mix allows reaction to be set up in room temperature and only requires the additions of primers and DNA template for the amplification.

Application:

- High-specificity PCR amplification
- High-throughput PCR
- Various cloning technologies
- Difficult amplification

Recommended Storage Condition: -20°C

Instructions:

	25 µl Reaction	50 µl Reaction	Final Concentration
I-5™ 2X High-Fidelity Master Mix	12.5 µl	25 µl	1X (see notes)
10 µM Primer A	1 µl	2 µl	400 µM
10 µM Primer B	1 µl	2 µl	400 µM
Template DNA	as required	as required	see notes
Water (ddH ₂ O)	up to 25 µl	up to 50 µl	

Thermocycling Conditions

3 Step PCR

Step	Temperature	Time	
Initial Denaturation	98°C	2 minutes	
Denaturation	98°C	10 seconds	25-35 cycles
Annealing	45°C - 68°C	10-15 seconds	
Extension	72°C	15-30 seconds / kb	
Final Extension	72°C	1-5 minutes	
	4°C	Hold	

2 Step PCR (see notes)

Step	Temperature	Time	
Initial Denaturation	98°C	2 minutes	
Denaturation	98°C	10-15 seconds	25-35 cycles
Combined Annealing & Extension	72°C	15-30 seconds / kb	
Final Extension	72°C	1-5 minutes	
	4°C	Hold	

Notes:

Recommended DNA Template addition

Genomic DNA	50-250 ng
Plasmid DNA	1 pg-10 ng
Viral DNA	1 pg-10 ng

2 Step PCR

Use of 2 Step PCR is recommended when the primer's T_m values are $>68^\circ\text{C}$

Mg^{2+}

The final concentration of Mg^{2+} in 1X I-5™ PCR Master Mix is 2 mM. Add additional Mg^{2+} as needed in 0.5 mM increments

Suggested the final Mg^{2+} concentration from 2 mM to 4 mM

PCR Product / Cloning

The 2X I-5™ PCR Master Mix results in PCR product with blunt ends Blunt-end cloning is recommended after PCR with this product

For T/A-cloning, the PCR product should be purified before A-addition