



Manual

Version 2.4

Product name: I-5™ Hotstart DNA Polymerase

Cat #: I5HD-100, I5HD-200, I5HD-OEM

Description:

I-5™ Hi-Fi DNA Hotstart polymerase is an ultra-fast and high-fidelity DNA polymerase. It provides robust amplifications of different templates including plasmids, BACS, genomic DNA, and lambda DNA. It allows the amplification of up to 8kb of human genomic DNA and up to 21kb of lambda DNA. Its has an extension speed of 1 kb / 5-10 seconds depending on template types. This allows the user to save time by speeding up PCR reactions and provides higher fidelity than *Taq* or PFU. The enzyme contains a Hotstart mechanism that inactivates the enzyme until it is heated. This allows the user to setup the PCR reactions at room temperature without worrying about primer dimers or non-specific preamplification.

Features:

- Fast – 4X faster than hot start *Taq*
- Robust – high inhibitor tolerance
- High yields – high efficiency
- Long PCR products

Recommended Storage Condition: -20 °C

Protocol:

Instructions

	25 µl Reaction	50 µl Reaction	Final Concentration
I-5™ 5X Buffer	5 µl	10 µl	1X (see notes)
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Primer A	1 µl	2 µl	400 µM
10 µM Primer B	1 µl	2 µl	400 µM
Template DNA	as needed	as needed	see notes
50mM MgCl ₂	as needed	as needed	see notes
I-5™ Enzyme	0.5 - 1 µl	1 - 2 µl	
Water (ddH ₂ O)	up to 25 µl	up to 50 µl	

Thermocycling Conditions

3 Step PCR

Step	Temperature	Time	25-35 cycles
Initial Denaturation	98°C	2 minutes	
Denaturation	98°C	10 seconds	
Annealing	55°C - 68°	10-15 seconds	
Extension	72°C	5-10 seconds / kb	
Final Extension	72°C	1-5 minutes	
	4°C	Hold	

2 Step PCR (see notes)

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

Notes

Recommended DNA Template addition	
Genomic DNA	50-250 ng
Plasmid DNA	1pg-10ng
Viral DNA	1pg-10ng

Mg ²⁺
The final concentration of Mg ²⁺ in I-5™ 5X Buffer is 2.0 mM Add additional Mg ²⁺ as needed in 0.5 mM increments Suggested the final Mg ²⁺ concentration ranges from 2 mM to 4 mM

2 Step PCR
Use of 2 Step PCR is recommended when the primers T _m value is >68°C