

# Manual

**Product name:** I-5™ Hi-Fi DNA Polymerase

**Cat #:** PDP-100, PDP-200, PDP-OEM

## Description:

I-5 Hi-Fi DNA polymerase is an ultra-fast and high-fidelity DNA polymerase. It provide robust amplification of from different templates including plasmids, BACS, genomic DNA, and lambda DNA. It allows for amplification of up to 8kb with human genomic DNA and up to 21kb with lambda DNA. Its has an extension speed of 1 kb / 5-10 seconds depending on template type. This allows the user to save time by speeding up PCR reactions and provides higher fidelity than *Taq* or *Pfu*.

## Features:

- Robust – maximal success and minimal optimization needed
- Fidelity – 50X greater than *Taq*
- High speed –10X faster than *Pfu*
- High yield
- Versatile – best for long or difficult templates

**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 <sub>2</sub>	as needed	as needed	see notes
I-5™ Enzyme	0.5 - 1 µl	1 - 2 µl	
Water (ddH <sub>2</sub> 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°C	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-10 ng
Viral DNA	1pg-10 ng

### Mg<sup>2+</sup>

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

### 2 Step PCR

Use of 2 Step PCR is recommended when the primer T<sub>m</sub> values are >68°C

### PCR Product / Cloning

The I-5™ Hi-Fi DNA Polymerase results in PCR products 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