SIGNALCHEM DIAGNOSTICS

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INSTRUCTIONS FOR USE

Product Name: High-Fidelity DNA Polymerase **Catalog # HF01-E21B**

Step 1. In a centrifuge tube, add the following components while keeping the reaction on ice:

2 µL (0.4 µM)	Forward Primer (10 µM)
2 µL (0.4 µM)	Reverse Primer (10 µM)
X µL	DNA Template
25 µL	PCR Buffer (including Mg2+, dNTPS)
Up to 50 µL	ddH_2O
1 µL	High Fidelity DNA Polymerase (1U/µL)

- Recommended amount for DNA templates:

50 ng-200 ng	Genomic DNA
10 pg-10 ng	Plasmid DNA
1-2.5 µL (<10% of final volume)	cDNA

- Final concentration of Mg^{2+} is 2mM and can be optimized to be between 0.2-0.5mM.

Step 2. Add 3% DMSO as a PCR additive to aid in the denaturing of templates with high GC contents.

Step 3. Run the appropriate thermal cycling settings:

The two-step protocol is fast with high specificity, medium PCR yield, and high detection rate. The three-step protocol is the average speed, specificity, and detection rate but has high PCR yield. The annealing gradient protocol is the slowest with high specificity, medium PCR yield, and high detection rate.

Two-Step Protocol:

Pre-denaturation	98°C	3 min	1 cycle
Denaturation	98°C	10 sec	30-35 cycles
Extension	68°C	30 sec/kb	
Final Extension	72°C	5 min	1 cycle

Annealing Gradient Protocol:

Pre-denaturation	98°C	3 min	1 cycle
Denaturation	98°C	10 sec	15, -1°C/cycle

Annealing Gradient	70-55°C	20 sec	
Extension	72°C	30 sec/kb	
Denaturation	98°C	10 sec	20 cycles
Annealing Gradient	55°C	20 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	1 cycle

Three-Step Protocol:

Pre-denaturation	98°C	3 min	1 cycle
Denaturation	98°C	10 sec	30-35 cycles
Annealing	60°C	20 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	1 cycle

Notes on three-step protocol:

- The pre-denaturation time can be increased to 5-10min for GC rich DNA templates.
- The annealing temp in the three-step protocol can be optimized and set to a temp gradient. The time can be adjusted between 10-30sec.
- The extension time can be extended to 60 sec/kb for complex templates.

Step 4. Store the PCR amplification products at - 20°C to prevent DNA degradation.