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INSTRUCTIONS FOR USE

Product Name: Ultra-Rapid Hot Start PCR Master Mix (With Dye) **Catalog # HS01-M29A**

Step 1. In a centrifuge tube, add the following components:

2.5 µL	Forward primer (10 µM)
2.5 µL	Reverse primer (10 µM)
25 µL	Ultra-Rapid Hot Start PCR Master Mix
X µL	DNA Template
Up to 50 µL	ddH_2O

- Note: Fully thaw and mix reagents prior to use.
- Recommended amount for DNA templates:

10-1000 ng	Genomic DNA or liquid *E. coli* culture
0.5-50 ng	Plasmid DNA or viral DNA
1-5 µL	cDNA (>10% of total reaction volume)

Step 2. Run the following thermal cycling settings:

Pre-denaturation	94°C	3 min	1 cycle
Denaturation	94°C	10 sec	28-35 cycles
Annealing	60°C	20 sec	
Extension	72°C	1-10 sec/kb	
Final Extension	72°C	5 min	1 cycle

- The annealing temp can be set 1-2°C lower than the theoretical value of the primer.
- Recommended extension speed depending on DNA template:

1 sec/kb	>1 kb Genomic DNA or >1 kb *E. coli* or <5 kb Plasmid DNA
3 sec/kb	1-3 kb Genomic DNA or 1-3 kb *E. coli*
5 sec/kb	5-10 kb Plasmid DNA
10 sec/kb	>3 kb Genomic DNA or >3 kb *E. coli* or >10 kb Plasmid DNA

Step 3. Store product at -20°C.