MediRes Corp.

USER GUIDE **Bi2M-2xpfu**

2X PCR Mix with High Fidelity Pfu

Product #: Bi2M-2xpfu
Quantity: 1 mL

Product Description

- The 2X PCR Mix is a concentrated solution of high fidelity *Pfu* DNA polymerase, dNTPs, and all other components required for PCR amplification, except DNA template and primers.
- The optimized *Pfu* DNA polymerase ensure efficient PCR reaction, high fidelity, and high specificity, and with quick annealing speed of 5-15s/kb.
- The 2X PCR Master Mix is premixed with a DNA loading dye, so the obtained product can be directly loaded on a gel.

Storage

- Store at -20 ^{0}C.

Components Provided

2X PCR Master Mix	1 mL

Important Preparatory Notes (PLEASE READ CAREFULLY)

1. It is recommended that all reaction components be assembled on ice.
2. Obtain DNA or cDNA of interest through a preferred DNA extraction and purification technique.
3. Prepare fresh dilutions of DNA or cDNA in either water or TE buffer.
4. We recommend using filtered nuclease-free pipette tips.

Protocol

1. Thaw **2X PCR Master Mix**, primers, and DNA. Once thawed, gently mix each component by inversion, pipetting, or mild vortexing.
2. Calculate the total volume required for the desired number of reactions, adding a 10% surplus, and prepare the assay mix of all components except DNA template accordingly. Thoroughly but gently mix by pipetting. Ensure liquid is collected at the bottom of the tube by brief centrifugation.

COMPONENT	20µL rxn	50µL rxn	FINAL CONCENTRATION
2X PCR Master Mix	10 µL	25 µL	1X
Forward Primer (10µM)	0.4 µL	1 µL	0.2 µM
Reverse Primer (10µM)	0.4 µL	1 µL	0.2 µM
DMSO (Optional)	0.6 µL	1.5 µL	3 %
Nuclease-Free Water	Add to 15 µL	Add to 45 µL	

3. Dispense assay mix into PCR tubes (Cat# PCR-02-FC), strip-tubes (Cat# PTS-200) or plates (Cat# WP-96-PCR-200-HS or WP-96-PCR-100-NS). For optimal results, maintain precise and consistent pipetting volumes, and minimize the formation of bubbles.
4. Add 5 µL DNA template to the PCR tubes or plates.

COMPONENT	20µL rxn	50µL rxn	FINAL CONCENTRATION
Template	Variable	Variable	Typically 1 to 10 ng (do not exceed 100 ng)
Nuclease-Free Water	Add to 5 µL	Add to 5 µL	

5. Seal tubes with flat, optically transparent caps, and seal plates with optically transparent film (Cat# PPH). Take care to seal plate edges and corners properly to prevent evaporation-related artifacts.
6. Briefly spin tubes or plates to eliminate bubbles and gather liquid (1 minute at 1,000 RCF).

7. Program the thermocycler as described below:

Step	Cycle #	Temperature	Time
Initial Denaturation	1	95℃	30 sec
Denaturation	25 – 35	95℃	15 – 30 sec
Annealing		55-68℃	15 – 30 sec
Extension		72℃	1 min/Kb
Final Extension	1	72℃	5 – 10 min
Hold	1	4℃	∞

Optimization of PCR Conditions

1. **Primers:** Typically, a satisfactory amplification outcome is achieved with a final concentration of 0.2µM. In instances of suboptimal reaction performance, primer concentration can be fine-tuned within the range of 0.2-1.0µM. Excessive primer concentration increases the probability of mispriming and the generation of non-specific PCR products.
2. **DNA Template:** The quantity of template added depends on the copy number of the target gene in the template solution, and the suitable amount of template addition is determined through gradient dilution. In a 20µL reaction system, the optimal amount of template DNA was found to be less than 10 ng (0.01-1 ng for both plasmid and viral DNA, and 0.1-100 ng for genomic DNA). When using cDNA from an RT-PCR reaction as a template, the addition amount should not exceed 10% of the total volume of the PCR reaction solution.
3. **Initial DNA Denaturation:** Template DNA needs to be completely denatured at the beginning of the PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95 ^{0}C is sufficient.
4. **Denaturation:** A DNA denaturation time of 30 seconds per cycle at 95 ^{0}C is normally sufficient. DNA template, which are GC-rich may need prolonged denaturation times.
5. **Primer Annealing:** The annealing temperature should be 5 ^{0}C lower than the melting temperature (Tm) of the primers. Annealing times of 30 seconds are normally sufficient. If nonspecific PCR products appear, the annealing temperature should be optimized via gradient or stepwise 1-2 ^{0}C increments.
6. **Extension:** The optimal extension temperature is 72 ^{0}C. The recommended extension time is 1 min/kb of expected product.
7. **Number of Cycles:** Typically, 25 to 35 cycles are sufficient to obtain adequate amounts of product. In cases where template concentration is low, an increased number of cycles may be required.