AmpFi Taq 2X PCR MasterMix

DM-AmpTaq400
Store at -20°C.

Description

The **AmpFi Taq 2X PCR MasterMix** is a ready-to-use solution that includes Taq DNA Polymerase, set in a specially designed buffer along with a gel loading dye. This Taq enzyme is capable of 5'-3' polymerase and 5'-3' exonuclease functions, but does not possess 3'-5' exonuclease activity. It generates amplicons that have 3'-dA overhangs. The PCR products created using this Taq are suitable for use with TA cloning vectors.

Product Component	Quantity
AmpFi Taq 2X PCR MasterMix*	400 rxn (10ml)

* Buffer contains final concentration of 1.5mM Mg^{2+}.

Protocol

1. Thoroughly thaw and mix individual components before use and assemble reaction on ice.

Component	Volume
AmpFi Taq 2X PCR MasterMix	25 µl
Forward Primer (10 µM)	1-2 µl
Reverse Primer (10 µM)	1-2 µl
Template DNA	Variable (200 ng genomic DNA)
Nuclease-free H_2O	Up to 50 µl

2. Gently mix the reaction components and briefly centrifuge and then transfer the tube to a thermal cycler. Use thermocycling conditions for standard PCR (1 kb template):

Step	Temperature	Time
Initial Denaturation**	94°C	3 min
25 – 35 Cycles	94°C 50-65°C*** 72°C	30 sec 30 sec 1min/kb**
Final Extension	72°C	5 min

** For most applications, an initial 3-minute denaturation step at 94°C is sufficient. Increase to 5 minutes for high-GC or difficult templates.

*** Adjust annealing temperature based on the primer melting temperature.

3. After PCR, maintain the reaction at 4°C or store at -20°C until use.
4. Analyze the amplification products by agarose gel electrophoresis.
5. Visualize by ethidium bromide or applicable staining.

Notes

- When dealing with challenging or high-GC content templates, employ a standard touchdown thermocycling program.
- In cases where the template concentration or the sample amount is low, adjust the total reaction volume to 25 µl, and scale down the volumes of other components proportionally.