AmpFi 2X qPCR MasterMix

DM-AmpqMM2500

Store at -20°C.

Description

AmpFi 2X qPCR MasterMix provides analysis of DNA samples for quantitative real-time in a convenient, reliable setup. This qPCR MasterMix contains Taq Polymerase and DNA Polymerase, providing rapid extension rates. This polymerase provides increased yields, and sensitivity, while optimizing reaction times by up to 70%, compared to wild-type Taq DNA polymerase.

The AmpFi 2X qPCR MasterMix possesses 5'-3' polymerase and 5'-3' exonuclease activities but does not have 3'-5' exonuclease activity. It generates amplicons with 3'-dA tails. qPCR products created with this MasterMix are compatible with TA cloning vectors. Additionally, the MasterMix includes a dye that is similar to both SYBR Green™ and EvaGreen™.

Product Component	**Quantity**
AmpFi 2X qPCR MasterMix	2500 rxn (25 ml)
ROX Reference Dye	240 µl

Protocol

The suggested quantity of ROX Reference Dye to be added to the MasterMix can differ based on the specific type of qPCR machine being used.

- No ROX equipment: Not needed.
- Low ROX equipment: 1 µl/1.25 ml or 22.5 µl/25 ml MasterMix.
- High ROX equipment: 11.5 µl/1.25 ml or 225 µl/25 ml MasterMix

1. Mix individual components before use and assemble reaction on ice.

Component	Volume
AmpFi 2X qPCR MasterMix*	10 µl
Forward Primer (10 µM)	0.5 µl
Reverse Primer (10 µM)	0.5 µl
Template DNA	Variable (100 ng genomic DNA)
Nuclease-free H2O	Up to 20 µl

*Reaction buffer contains 1.5 mM Mg^{2+}

2. Gently mix the reaction components, and briefly centrifuge. Use thermocycling conditions:

Step	Temperature	Duration (Standard)	Duration (Fast)	Cycles
Enzyme Activation	95°C	3 min	3 min	1
Denaturation	95°C	15 sec	1 sec	40
Annealing/Extension	60°C	1 min	10 sec	
Melting Curve	Refer to specific guidelines for the instrument used			

Notes

- The specialized buffer in this formulation enhances yields, sensitivity, and specificity beyond what is typically achieved with wild-type Taq polymerase.
- It's best to begin the qPCR immediately after preparing the reaction mixture. If that's not feasible, store the mixture on ice until you're ready to start the qPCR.
- For miRNA cDNA templates or other relevant applications, employ the standard thermocycling conditions.