

AmpFi RT MasterMix

DM-AmpRT100

Store at -20°C.

Description

AmpFi RT MasterMix was designed to perform simple, reliable, and reproducible first-strand cDNA synthesis. The superior quality cDNA produced using this kit is suitable for various downstream applications.

Components:

- AmpFi Reverse Transcriptase
- Ribonuclease Inhibitor
- Temperature-sensitive DNase
- dNTPs
- Oligo (dT)s and Random Primers.

Product Component	Quantity
AmpFi RT MasterMix	100 rxn (400 µl)
Nuclease-Free H ₂ O	1.0 ml

Protocol

1. Fully thaw and thoroughly mix each component before use, and then assemble the reaction while keeping it on ice.

Component	Volume
AmpFi RT MasterMix	4 µl
Total RNA or poly(A) + mRNA	Variable (1 ng - 2 µg/rxn)
Nuclease-Free H ₂ O	up to 20 µl

2. Gently mix the reaction and briefly centrifuge.
3. Incubate the mixture at 37°C for 15 minutes, followed by 60°C for 10 minutes.
4. Optional: Terminate the reaction by heating at 95°C for 3 minutes, then cool it down on ice. The freshly synthesized first-strand cDNA can be used immediately in downstream applications, or stored long-term at -20°C.

Notes

- Extending the incubation period at 37°C from 15 minutes to 30 minutes can enhance the removal of genomic DNA (gDNA).
- Follow the manufacturer's protocol to add E. coli RNase H, which helps in eliminating RNA that is complementary to the cDNA.
- Although both poly(A) + mRNA and total RNA are suitable for first-strand cDNA synthesis, using poly(A) + mRNA may result in higher yields and better purity of the final products.