AmpFi cDNA Synthesis Kit

DM-AmpSyn100

Store at -20°C.

Description

AmpFi cDNA Synthesis Kit was designed to work on the most challenging RNA samples due to its outstanding thermostability at 60-72ºC.

- **High processivity and sensitivity:** cDNA synthesis of full-length cDNA in half of the time of the kits in the market.
- **Sensitive**: Reverse transcribe low abundance or degraded RNA.
- **Resistance** to contaminating reaction inhibitors.
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This kit contains a ribonuclease inhibitor, which improves oxidation resistance. It is stable even under very low concentrations of DTT (< 1 mM).

This kit includes a complete range of reagents required to produce high-quality cDNA, offering great flexibility in terms of priming methods and reaction optimization. It contains both random primers and oligo(dT), providing options for general priming strategies or as alternatives to gene-specific primers.

Product Component	Quantity
AmpFi Reverse Transcriptase	100 rxn (100 µl)
5X RT Buffer	400 µl
Oligo(dT) (10 µM)	100 µl
Random Primers (10 µM)	100 µl
dNTP (10 mM)	100 µl
Nuclease-Free H_2O	2 x 1.0 ml

Protocol

RT reactions need to be set up in an environment free of RNase. It is advisable to use separate pipettors dedicated for PCR and to employ aerosol-resistant barrier tips.

1. Before using, ensure that each component is completely thawed and well mixed. Then, proceed to assemble the reaction while keeping it on ice.

Component	Volume
5X RT Buffer	4 µl
dNTP	1 µl
Primers	1 µl
Total RNA or poly(A) + mRNA	Variable (1 ng - 2 µg/rxn)
AmpFi Reverse Transcriptase	1 µl
Nuclease-free H_2O	up to 20 µl

2. Gently mix the reaction and briefly centrifuge.
3. Perform cDNA synthesis by incubating for 15 minutes at 60°C.
4. Optional: To terminate the reaction, heat at 85°C for 5 minutes. Then cool it down on ice. The newly synthesized first-strand cDNA is now prepared for immediate use in downstream applications or can be stored long-term at -20°C.

Notes

- You can use either poly(A) + mRNA or total RNA for first-strand cDNA synthesis, though poly(A) + mRNA tends to yield higher amounts and purer final products.
- For synthesizing longer transcripts (over 9 kb), increase yields by incubating at 60°C for 30-50 minutes.
- Ensure RNA samples are devoid of genomic DNA contamination.
- The proportion of Random Primers to RNA is crucial for determining the average length of the synthesized cDNA. A higher ratio of Random Primers to RNA typically generates a greater yield of shorter cDNA (around 500 bp), while a lower ratio favors the production of longer cDNA. Given the lower annealing temperature of Random Primers, allow for primer annealing at 25°C for 10 minutes before proceeding with reverse transcription.
- To eliminate RNA that is complementary to the cDNA, incorporate E. coli RNase H as directed by the manufacturer’s protocol.