cDNA Synthesis Kit

Catalogue No.:abx460012

cDNA Synthesis Kit for optimal cDNA synthesis from a variety of RNA samples over a wide temperature range. The kit contains a M-MLV reverse transcriptase with extended thermostability and half-life and contains proprietary mutations for reduced RNase H activity.

Kit Components:

- Enzyme Mix: 100 µl
- 2X Reaction Mix: 500 µl
- Oligo dT_{20} Primer (50 µM): 50 µl
- Random Hexamer Primer (50 ng/µl): 50 µl
- Nuclease-Free Water: 1 ml

Materials Required But Not Provided:

- Vortex Mixer
- Microcentrifuge
- Pipettes and Pipette Tips
- PCR Tubes
- Ice Water Bath
- Temperature-Controlled Water Bath (or equivalent)

Target:	cDNA Synthesis Kit
Tested Applications:	PCR
Storage:	Store at -20 °C for up to 18 months. Avoid repeated freeze/thaw cycles.
Buffer:	Enzyme: 20 mM Tris-HCl, pH 7.4, 0.1 M NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% NP-40 and 50% glycerol.
Concentration:	200 U/µl
Note:	THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

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Directions for use:

Assay Procedure:

1. Mix and heat the RNA Template, 2X Reaction Mix, and Primers to 65 °C for 5 minutes. Allow the RNA Template to stand in an ice bath for at least 1 minute.

2. Add the following components to a PCR tube on ice:

Component	**Volume**
RNA Template	Variable 10 pg - 2 µg total RNA or 10 pg - 500 ng mRNA)
2X Reaction Mix	10 µl
Primers	1 µl
Enzyme Mix	2 µl (200 U)
RNase Inhibitor	1 µl (20-40 U)
Water	Variable, up to 20 µl
Total Volume	**20 µl**

3. Mix by gently pipetting up and down.

4. Close the lid on the tube and incubate in a temperature-controlled water bath at 55 °C for 50 minutes for the extension step.

Note: the optimal temperature for extension is likely between 42-60 °C.

5. Incubate the tube at 70 °C for 15 minutes to inactivate the Reverse Transcriptase before amplification.

Notes:

• Avoid cross-contaminating the RNA template (total RNA, synthetic RNA transcript, poly(A) + mRNA) with DNA.

• Recommended primers (per 20 µl reaction):
  - 2.5 µM of oligo(dT) - anneal to 3'-poly(A) + mRNA
  - 2.5 ng/µl of random primers - anneal at non-specific sites of RNA templates
  - 2 µM of gene-specific primers

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