Cat. No. AECD1250-50

For Research Use Only

Azma cDNA Synthesis Kit

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DESCRIPTION

The Azma cDNA Synthesis Kit is a complete system for efficient synthesis of first strand cDNA from mRNA or total RNA templates. The kit uses Thermo-resistant H Minus M-MuLV Reverse Transcriptase, which has no RNase H activity. Therefore, degradation of RNA does not occur during first strand cDNA synthesis, resulting in higher yields of full-length cDNA from long templates compare to other reverse transcriptases. The enzyme maintains active at 42-50°C and is suitable for synthesis cDNA from RNAs having a high degree of secondary structure.
This cDNA synthesis kit is readily compatible with various cDNA-dependent downstream applications.

Components of AZMA cDNA Synthesis Kit	**Cat. No. AECD1250-50**
RT Enzyme	25 µl
5X RT Buffer	125 µl
Oligo dT Primer (ready to use)	50 µl
Random Hexamer Primer (ready to use)	50 µl
dNTP Mixture (ready to use)	50 µl
Nuclease Free Water	200 µl

STORAGE

All components of the kit should be stored at **-20**°C.

IMPORTANT NOTES

- **Avoiding ribonuclease contamination**

RNA purity and integrity are essential for synthesis of full-length cDNA. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment.
General recommendations to avoid RNase contamination:

- DEPC-treat all tubes and pipette tips to be used in cDNA synthesis or use nuclease-free labware.
- Wear gloves when handling RNA and all reagents, as skin is a common source of RNases.
- Keep all kit components tightly sealed when not in use. Keep all tubes tightly closed during the reverse transcription reaction.

- **Make Master mix A and B in a work station and in a separate area from Extraction Room.**
- **No template negative control (NTC) is important to assess for reagent contamination.**
  The NTC reaction contains every reagent for the reverse transcription reaction except for RNA template.
- **PRODUCT USE LIMITATION**
  This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

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PROTOCOL

Note: After thawing, gently vortex and briefly centrifuge the components of the kit. Store on ice.

1) Make **Master Mix A** with the following order in a **2 ml** DNase and RNase free tube:

A1	Add **1 µl** of **Oligo dT primer** per sample.
A2	Add **1 µl** of **Random Hexamer primer** per sample.
A3	Add **1 µl** of **dNTP Mixture** per sample.

2) After mix and briefly spin down the **Master Mix A**, aliquot **3 µl** of it per sample into **0.5 ml** DNase and RNase free **reaction tubes**. Keep on ice until used.
3) Make **Master Mix B** in a **new 2** ml DNase and RNase free tube with the following order:

B1	Add **0.5 µl** of **RT enzyme** per sample.
B2	Add **2.5 µl** of **5X RT Buffer** per samples.
B3	Add **4 µl** of **Nuclease Free Water** per sample.

Note: Keep **Master Mix B** on ice until used.

4) **Add 10 µl** of **total RNA** or **mRNA** to every **0.5 ml reaction tube** (contained Master Mix A).
5) **Incubate** at **65ºC** for **5 minutes** and then incubate at **4 ºC** for **1 minute**. Briefly spin down the mixture.
6) **Add 7 µl** of **Master mix B** to every **0.5 ml reaction tube**.
7) **Incubate** at **47** ºC for **60** minutes and then at **80 ºC** for **8 minutes**.
8) Briefly spin down the mixture and store at **-20 ºC**.

Note: The synthesized cDNA can be directly used for downstream application or stored at **-20 ºC**.

Note: The volume of total cDNA would be **20 µl**.

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