4× Reverse Transcription Master Mix

Catalog No.: A0010T

Description

The ***EZ*Bioscience**® **4× Reverse Transcription Master Mix** is a ready-to-use premixed reagent that contains all the reagents necessary to synthesize first strand cDNA from total or poly(A)+ RNA using Reverse Transcriptase. Reaction products are applicable to subsequent PCR, qPCR and PCR cloning. The mix contains Reverse Transcriptase, RNase Inhibitor, optimized buffer system, and dNTPs. Oligo dT18 and Random Hexamer are supplied in separate tubes as primer.

The Reverse Transcriptase in this Mix is a genetic engineered enzyme based on M-MLV (RNase H-) reverse transcriptase. The reverse transcriptase lacking RNase H activity is suitable for preparing full-length cDNA. And the multiple site-mutations of Reverse Transcriptase can obviously increase its affinity to RNA templates and its strand extending ability, which make reverse transcription reaction more efficient. So more full-length cDNAs, and also, cDNAs as long as 20 kb can be synthesized. Moreover, this transcriptase is rather resistant to common reverse transcriptase inhibitors. This product is also very suitable for reverse transcription using plant tissue RNA.

Components

Components	A0010T (100 Rxns)	A0010T-L (500 Rxns)
4× RT Master Mix	550 µl	550 µl × 5 tubes
Oligo dT18 (40 µM)	110 µl	110 µl × 5 tubes
Random Hexamer (80 µM)	110 µl	110 µl × 5 tubes
Nuclease free ddH_2O	1 ml	1 ml × 5 tubes

Storage

Store at -20°C.

Caution

Avoid RNase contamination

Please keep the environment of experiment clean. Clean gloves and mask should be worn during the experiment. Centrifuge tubes, tips and other supplies used in the experiment must be RNase-free.

Protocol

Reverse Transcription

1a. **For microRNA, lncRNA and circRNA,** combine the following components in a RNase-free centrifuge tube, then mix gently with a pipette and centrifuge the mixture briefly to the bottom of the tube.

Components	20 µl Reaction
Total RNA	1 ug (100 ng ~ 2 µg)
or Poly(A)+ RNA	50 ng (10 pg ~ 500 ng)
4× RT Master Mix	5 µl
Target Specific primer (100 nM)	1 µl
Nuclease free ddH_2O	to 20 µl

1b. **For mRNA**, combine the following components in a RNase-free centrifuge tube, then mix gently with a pipette and centrifuge the mixture briefly to the bottom of the tube (Random Hexamer is not necessary for the reaction. However, to get better qPCR results, it is recommended to add the Random Hexamer).

Components	20 µl Reaction
Total RNA	1 ug (100 ng ~ 2 µg)
or Poly(A)+ RNA	50 ng (10 pg ~ 500 ng)
4× RT Master Mix	5 µl
Oligo dT18	1 µl
Random Hexamer (optional)	1 µl
Nuclease free ddH_2O	to 20 µl

2. Perform the reverse transcription reaction at 42°C for 15 minutes and 95°C for 30 seconds.

3. The reverse transcription reaction product can be directly used in PCR applications, but the cDNA is recommended to dilute and mix thoroughly before use (The specific dilution factor depends on the abundance of gene expression. Generally, cDNA is diluted 5 ~ 10 times). If the qPCR experiment is not performed immediately, it is recommended to store at -80°C for long-term storage. Avoid repeated freeze-thaw cycles.