4× Reverse Transcription Master Mix

Catalog No.: A0010

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, dNTPs; Oligo dT18 and random primer as primer. The random primer in the Mix can help get better Ct values.

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. Moreover, this transcriptase is rather resistant to common reverse transcriptase inhibitors. This product is also very suitable for reverse transcription using plant tissue RNA.

When using this kit, 4× RT Master Mix should be added to total RNA template, as recommended in the protocol, and add ddH_2O to a total volume of 20 µl. Then, mix well and run the RT reaction. The RT reaction is recommended at 42°C for 15 minutes and 95°C for 30 seconds.

Components

Components	A0010 (100 Rxns)	A0010-L (500 Rxns)
4× RT Master Mix	550 µl	550 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

1. Combine the following components in a RNase-free centrifuge tube.

Components	20 µl Reaction
4× RT Master Mix	5 µl
Total RNA	1 ug (100 ng ~ 2 µg)
or Poly(A)+ RNA	50 ng (10 pg ~ 500 ng)
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.