4× Reverse Transcription Master Mix (with gDNA Remover)

Catalog No.: A0010GQ

Description

The ***EZ*Bioscience**® **4× Reverse Transcription Master Mix (with gDNA Remover)** is a kit suitable for real-time RT-PCR (RT-qPCR) that contains a gDNA Remover which can effectively eliminate the contamination of genomic DNA (gDNA) or other double stranded DNA during the analysis of gene expression.

To accurately analyze gene expression, it is necessary to detect cDNA in samples without contaminating DNA. To avoid amplification of gDNA, primers can be designed on different exons spanning introns. However, there may be cases where a suitable primer cannot be designed, as with a gene with a single exon or a gene without a long intron. Also, it may be difficult to avoid unexpected amplification from gDNA due to non-specific amplification or the existence of pseudo-genes. Moreover, some labs are heavily contaminated by the PCR products of previous tests. The kit offers a potent gDNA Remover that can eliminate double stranded DNA in RNA sample in 5 minutes at room temperature without loss of RNA. Then first strand cDNA is synthesized by adding the 4× RT Master Mix. Reaction products are applicable to subsequent PCR, qPCR.

The mix contains Reverse Transcriptase, RNase Inhibitor, optimized buffer system, dNTPs, and Oligo dT18 and Random Hexamer as primers. The Reverse Transcriptase in this Mix is a genetic engineered enzyme based on M-MLV (RNase H-) reverse transcriptase. 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.

Components

Components	A0010GQ (100 Rxns)	A0010GQ-L (500 Rxns)
4× RT Master Mix	550 µl	550 µl × 5 tubes
gDNA Remover	220 µl	220 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

gDNA Remover treatment of RNA

1. Add 1 µg total RNA (100 ng ~ 2 µg adjustable) or 50 ng Poly(A)+ RNA (10 pg ~ 500 ng adjustable) to a new RNase-free centrifuge tube. Add 2 µl gDNA Remover to the RNA, pipette up and down for 10 times to mix thoroughly. Incubate at room temperature (19 ~ 27°C) for 5 minutes.

Reverse Transcription

2. Add components to the gDNA Remover-treated RNA according to the following table, then mix gently with a pipette and centrifuge the mixture briefly to the bottom of the tube.

Components	20 µl Reaction
gDNA Remover treated RNA	X µl
4× RT Master Mix	5 µl
Nuclease free ddH_2O	up to 20 µl

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

4. 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.