4× Reverse Transcription Master Mix (with gDNA Remover)

Catalog No.: A0010G

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. In order 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 and primers. Reaction products are applicable to subsequent PCR, qPCR and PCR cloning. The template could be mRNA, microRNA, lncRNA, circRNA, etc.

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.

Components

Components	A0010G (100 Rxns)	A0010G-L (500 Rxns)
4× RT Master Mix	550 µl	550 µl × 5 tubes
Oligo dT18	110 µl	110 µl × 5 tubes
Random Hexamer	110 µl	110 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 of total RNA (10 pg ~ 2 µg) or 50 ng Poly(A)+ RNA (10 pg ~ 500 ng) 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 (Optional: the RNA could be heated at 85°C for 1 min and put on ice immediately, before adding the RT reagents to it).

Reverse Transcription

2a. **For microRNA, lncRNA and circRNA**, 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
Target Specific primer (100 nM)	1 µl
Nuclease free ddH_2O	to 20 µl

2b. **For mRNA**, 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 (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
gDNA Remover treated RNA	X µl
4× RT Master Mix	5 µl
Oligo dT18	1 µl
Random Hexamer (optional)	1 µl
Nuclease free ddH_2O	to 20 µl

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

The cDNA products can be used qPCR in reactions immediately, or stored at -80°C for long-term storage. Avoid repeated freeze-thaw cycles.