Color Reverse Transcription Kit (with gDNA Remover)

Catalog No.: A0010CG

Description

The ***EZ*Bioscience**® Color Reverse Transcription Kit (with gDNA Remover) is a new-generation fast reverse transcription kit containing high-efficiency DNase for the removal of genomic DNA and high-stability blue dyes, which has higher reverse transcription efficiency than the previous generation.

To accurately analyze gene expression, it is necessary to detect cDNA in samples without contaminating DNA. To avoid amplification of genomic DNA (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 is suitable for real-time RT-PCR (RT-qPCR) that contains a gDNA Remover which can effectively eliminate the contamination of gDNA or other double stranded DNA in RNA samples at room temperature for 5 minutes without loss of RNA during the analysis of gene expression. Then the first strand of cDNA is synthesized by adding the 4× RT Master Mix and primers. Reaction products are applicable to subsequent PCR, qPCR and PCR cloning. The RNA sample could be mRNA, microRNA, lncRNA, circRNA, etc.

The 4× RT Master Mix of the kit contains reverse transcriptase, RNase Inhibitor, optimized buffer system, dNTPs, and blue dye. blue dye in the 4× RT Master Mix could avoid of pipetting errors. It can be used with the ***EZ*Bioscience**® *Color SYBR Green qPCR Mix (A0012, A0012-R1, A0012-R2)*, which is supplemented with an inert **red dye**. Mixing cDNAs, primers and other components with the ***EZ*Bioscience**® Color SYBR Green qPCR Mix together in a qPCR reaction turns the solution into **purple**, which provides a visual aid when pipetting and decreases the risk of pipetting errors during reaction setup, especially when using white reaction vessels. And the dyes do not affect the specificity or sensitivity of qPCR reactions. So, these two kinds of kits are recommended to use together to get optimal results.

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. At the same time, this kit uses the latest optimized reaction system to further improve the reverse transcription efficiency. This product is also very suitable for reverse transcription using plant RNA.

Components

Components	A0010CG (100 Rxns)	A0010CG-L (500 Rxns)
gDNA Remover	220 µl	220 µl × 5 tubes
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
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, pipetting 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***, set up the following mixture according to the table below, mix gently

with a pipette.

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**, set up the following mixture according to the table below, mix gently with a pipette (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 Polyadenylation and reverse transcription at 42°C for 15 minutes, 95°C for 30 seconds.

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