

# Color Reverse Transcription Kit (with gDNA Remover)

Catalog No.: A0010CGQ

## Description

The **EZBioscience**<sup>®</sup> 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 ddH<sub>2</sub>O. Reaction products are applicable to subsequent PCR, qPCR.

The 4× RT Master Mix contains reverse transcriptase, RNase Inhibitor, optimized buffer system, dNTPs, Oligo dT18 and Random Hexamer as primers, and blue dye. blue dye in the 4× RT Master Mix could avoid of pipetting errors. It can be used with the **EZBioscience**<sup>®</sup> 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 **EZBioscience**<sup>®</sup> 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. 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, the 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	A0010CGQ (100 Rxns)	A0010CGQ-L (500 Rxns)
gDNA Remover	220 µl	220 µl × 5 tubes
4× RT Master Mix	550 µl	550 µl × 5 tubes
Nuclease free ddH <sub>2</sub> O	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 (10 pg ~ 2 µg adjustable) or 200 ng Poly(A)<sup>+</sup> 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. 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
Nuclease free ddH <sub>2</sub> O	up 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.