TOOLSQuant II Fast RT Kit

For first-strand cDNA synthesis and removal of gDNA

Contents

Introduction

TOOLSQuant II Fast RT Kit is designed for high-efficiency first-strand cDNA synthesis and can rapidly remove genomic DNA (gDNA). This kit contains a gDNA eraser that effectively removes gDNA (42°C, 3 min). Its innovative RT enzyme enables the synthesis of first-strand cDNA to be completed within only 15 min (42°C). This product also has a high affinity for RNA, thus allowing for the delivery of efficient and sensitive reverse transcriptions of any template—such as GC-rich and complicated secondary structures of template RNA—which results in high yields of cDNA.

Application

RT-PCR, quantitative PCR (qPCR), cDNA library construction, serial analysis of gene expression (SAGE), and primer elongation

Important Notes

1. For optimal results, the use of 50 ng to 2 μg of RNA is recommended. For RNA >2 μg, scale up the reaction linearly to the appropriate volume.
2. Assemble the reactions on ice to avoid the degradation of RNA.
3. To aid the denaturing of RNA with a high GC concentration or secondary structures and to ensure that the primer anneals to the target efficiently, incubate the template RNA at 65°C for 5 min and then place the samples on ice before immediately performing the reverse transcription.
4. The Oligo-dT Primer and Gene Specific Primer can both be used for reverse transcription.
   Reaction quantity: Oligo-dT Primer, 50 pmol/20 μL; Gene Specific Primer, 5 pmol/20 μL
5. When using the Gene Specific Primer, reverse transcription for 15 min at a temperature of 42°C is recommended. The temperature can be set to 50°C if nonspecific PCR products are found.
6. The reaction volume can be proportionally scaled up.

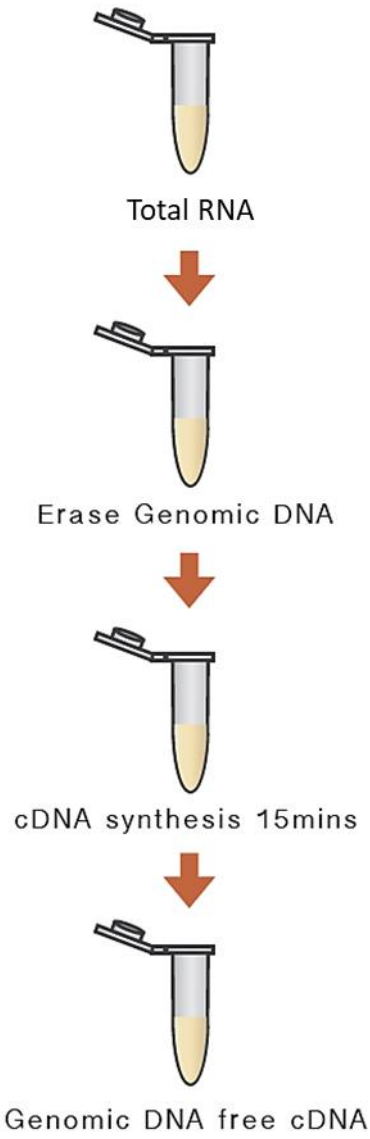
Kit Contents

Contents	KRT-BA06 (25 preps)	KRT-BA06-2 (100 preps)
5×gDNA Eraser	50 μL	200 μL
RT Primer Mix	50 μL	200 μL
RT Enzyme Mix	25 μL	100 μL
10×Fast RT Buffer	50 μL	200 μL
RNase- Free ddH_2O	1 mL	2x 1 mL

Storage

A TOOLSQuant II Fast RT Kit can be stored at −20 °C for up to 12 months.

Workflow



Protocol

A total reaction volume of 20 µL corresponds to 50 ng to 2 µg of RNA.

1. Thaw the template RNA on ice. Thaw 5 × gDNA Eraser, 10 × Fast RT Buffer, the RT Primer Mix, and RNase-Free ddH_2O at room temperature and then immediately place them on ice. Briefly vortex and centrifuge all solutions to collect the residual liquid from the sides of the tubes before use.

   Perform the following steps on ice. To ensure equivalence between each reaction, for each reaction tube, make a master mix and then aliquot the mix.

2. Prepare the gDNA removal reaction on ice according to Table 1. Mix thoroughly and carefully by briefly vortexing and centrifuging the mixture. Incubate for 3 min at 42°C and then store on ice.

Table 1. gDNA removal reaction components

Component	1 rxn
5×gDNA Eraser	2 µL
Total RNA template (50 ng- 2 µg)	-
RNase-Free ddH_2O	Up to 10 µL

3. Prepare the reverse transcription reaction according to Table 2.

Table 2. Reverse transcription reaction components

Component	1 rxn
10×Fast RT Buffer	2 µL
RT Enzyme Mix	1 µL
RT Primer Mix	2 µL
RNase-Free ddH_2O	Up to 10 µL

4. Thoroughly mix the gDNA removal reaction and reverse transcription reaction.
5. Incubate each reaction at 42°C for 15 min.
6. Terminate each reaction at 95°C for 3 min and then place it on ice. The synthesized cDNA should be stored at −20°C for use in subsequent experiments.

Note: Reverse transcriptase synthesizes first-strand cDNA from template RNA. The purity and integrity of the template RNA affect the quality of the reverse-transcripted cDNA product. If the template RNA contains RNase, cDNA yields will be low. Protein, salt, ethanol, and phenol residues in template RNA can also affect the quality of reverse transcription.

For subsequent qPCR experiments, the volume of cDNA should not exceed 10% of the total qPCR reaction volume (for a 50-µL qPCR reaction mix, cDNA volume should not exceed 5 µL).