TIANGEN®

FastKing RT Kit (With gDNase)

21 min high-efficient reverse transcription with gDNA cleaning up

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Cat. no. 4992223/4992224/4992250

Kit Contents

Contents	4992223 25 rxn	4992224 100 rxn	4992250 1000 rxn
5 × gDNA Buffer	50 µl	200 µl	10 × 200 µl
FQ-RT Primer Mix	50 µl	200 µl	10 × 200 µl
FastKing RT Enzyme Mix	25 µl	100 µl	10 × 100 µl
10 × King RT Buffer	50 µl	200 µl	10 × 200 µl
RNase-Free ddH_2O	1 ml	2 × 1 ml	10 ×2 × 1 ml
Handbook	1	1	10 × 1

Storage

FastKing RT Kit should to be shipped with dry ice and can be stored at -20°C for up to 12 months.

Introduction

FastKing RT kit is an efficient, rapid and genomic DNA cleaning up reverse transcription system. This product contains gDNase which can remove genomic DNA by incubation at 42°C, 3 min to protect the total RNA from genomic DNA interference. King RT Enzyme in RT Enzyme Mix provides a high-efficient reverse transcription with 42°C, 15 min. With a special modified hydrophobic motif, King RTase gets a significant affinity for RNA and facilitates transcription through of RNA templates, and enables read-through of templates with high GC content or complex secondary structures.

Product Features

High RT efficiency: RT efficiency higher than 95%

Simple and easy to operate: Simple reaction set up, first strand cDNA can be synthesis within 21 minutes.

Read complex template: Enables read-through of templates with high GC content or complex secondary structures.

High sample universality: Cabable for high impurity content RNA templates and RNA templates with different species.

Excellent capability: Could be co-used with qPCR product with high sensitivity and stability.

Important Notes

1. This protocol is optimized for use with 50 ng to 2 μg of RNA. With >2 μg RNA, scale up the reaction linearly to the appropriate volume.
2. Operate on ice to minimize the risk of RNA degradation.
3. Separate denaturation and annealing steps are generally not necessary. However, for some RNAs with a high degree of secondary structure, a denaturation step may be desired. If so, denature the RNA in RNase-free water before reaction setup: incubate the RNA for 5 min at 65°C, then place immediately on ice.
4. For some transcripts, oligo-dT primers or Gene Specific Primer is possible. The final primer concentrations are as follow: Oligo-dT Primer 50 pmol/20 μl reaction system; Gene Specific Primer 5 pmol/20 μl reaction system.

5. When using Gene Specific Primers, we recommend a reverse transcription temperature of 42°C, 15 min. Raise the reaction temperature to 50°C will be helpful when non-specific amplification appeared.
6. Reverse transcription system could be scale up when necessary.

Protocol

The protocol is optimized for use with 50 ng to 2 μg of RNA.

1. Thaw template RNA on ice. Thaw 5 × gDNA Buffer, FQ-RT Primer Mix, 10×King RT Buffer, RNase-Free ddH_2O at room temperature (15-25°C). Place on ice immediately after thawing. Mix each solution by vortex, and centrifuge briefly to collect residual liquid from the sides of the tubes.

   The following steps are all requires operate on ice to guarantee the precision of reaction set up. Reagent mix should be set up before every reaction, then aliquot the mix to each tube.

2. Prepare a fresh master mix to clean up genomic DNA according to Table 1. Mix thoroughly and carefully for no more than 5 sec. Centrifuge briefly to collect residual liquid from the sides of the tube, incubate the mixture for 3 min at 42°C then store on ice.

Table 1. gDNA Clean-up Reaction Components

Component	Volume/Reaction
5× gDNA Buffer	2 μl
Total RNA	-
RNase-Free ddH_2O	Up to 10 μl

3. Prepare a fresh master mix for revise transcription according to table 2. Mix thoroughly and carefully for no more than 5 sec. Centrifuge briefly to collect residual liquid from the walls of the tube.

Table 2. Reverse-Transcription Reaction Components

Component	Volume/Reaction
10× King RT Mix	2 μl
FastKing RT Enzyme Mix	1 μl
FQ-RT Primer Mix	2 μl
RNase-Free ddH_2O	Up to 10 μl

4. Add revise transcript mixture into the liquid getting from step 2, mix thoroughly.
5. Incubate for 15 min at 42°C.
6. Incubate for 3 min at 95°C, then put on ice. The cDNA could be used in following experiments or stored in low temperature.

RNA template quality control

Reverse transcriptase takes RNA as template to synthesize the first strand cDNA, so the quality of template RNA directly affects the result of reverse transcription.

1. template integrity: the integrity of template RNA is very important for reverse transcription. If RNA template contains RNase, the template RNA will be degraded and the amount of cDNA product will be decreased or even no cDNA product.
2. template purity: if RNA template contains protein, ions, EDTA, ethanol, phenol and other impurities, the activity of the enzyme will be inhibited or changed and eventually affects the reverse transcriptional results. If genomic DNA is contained, the accuracy of subsequent experiments will be affected.
3. template addition: this protocol is optimized for use with 50 ng to 2 μg of RNA. With >2 μg RNA, scale up the reaction linearly to the appropriate volume.