TIANGEN®

FastKing gDNA Dispelling RT SuperMix

18 min high-efficient reverse transcription and gDNA cleaning up in one step

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FastKing gDNA Dispelling RT SuperMix

Cat. no. GKR118

Kit Contents

Contents	GKR118-01 25 rxn	GKR118-02 100 rxn	GKR118-03 1000 rxn
5 × FastKing-RT SuperMix	100 µl	400 µl	10 × 400 µl
RNase-Free ddH_2O	1 ml	2 × 1 ml	10 × 2 × 1 ml
Handbook	1	1	10 × 1

Storage

Store at -30~-15°C for up to one year.

Introduction

FastKing gDNA Dispelling RT SuperMix provides a rapid, stable and efficient method of cDNA synthesis which is perfect for downstream two-step Real Time PCR. 5 × FastKing-RT SuperMix contains all the required reagents of RT-PCR (include FastKing RT Enzyme, RNase Inhibitor, Random primers, Oligo dT Primer, dNTP Mixture and Reaction Buffer), and a special heat-sensitive DNase to efficiently remove genomic DNA without interfering with cDNA. Reaction could be started immediately right after the addition of template RNA and RNase-Free ddH_2O.

FastKing RT Enzyme in the SuperMix provides a high-efficient reverse transcription with 42°C, 15 min. With a special modified hydrophobic motif, FastKing Rtase gets a significant affinity for RNA and facilitates the efficiency and speed of the reaction, and enables read-through of templates with high GC content or complex secondary structures.

Product Features

Simple reaction setup: This product is premix-format, the reaction could be started right after the addition of RNA template and ddH_2O.

High performance reverse transcription: 95% of RNA template could be reverse transcribed to cDNA.

Short reaction time: gDNA can be removed and cDNA be synthesized together in one step within 15 min at 42°C.

Good for complex template: This product can be used for the reverse transcription of RNA template which has complex secondary structure and high GC content.

High compatibility for downstream analysis: This product could be co-used with many type of qPCR product for analysis with high sensitivity and stability.

Application Range

RT-PCR; RT-qPCR; cDNA library construction; SAGE

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 all the experimental process on ice to minimize the risk of RNA degradation.
3. Separate denaturation and annealing steps are generally not necessary. However, for some RNA templates with complex secondary structure, a denaturation step is recommended. If so, denature the RNA before reaction setup: incubate the RNA for 5 min at 65°C, then place immediately on ice.
4. Reverse transcription system could be scaled up when necessary.

Protocol

Synthesize first-strand cDNA with FastKing gDNA Dispelling RT SuperMix, this protocol is optimized for the setup of a 20 µl reaction with the RNA template amount range from 50 ng to 2 µg.

1. Thaw RNA template on ice. Thaw 5 × FastKing-RT SuperMix and RNase-Free ddH_2O at room temperature (15-30°C), and then place them on ice immediately after thawing. Mix each solution by vortex, and centrifuge briefly to collect residual liquid from the sides of the tubes.

   Please operate the following steps on ice to guarantee the accuracy of reaction setup. Please setup the reaction to mix and then make aliquots.

2. Setup the reaction according to the following table.

Component	Volume
5 × FastKing-RT SuperMix	4 µl
Total RNA	50 ng-2 µg
RNase-Free ddH_2O	Up to 20 µl

3. Start the reaction according to the following table.

Temperature	Time	Comment
42°C	15 min	gDNA removing and reverse transcription
95°C	3 min	Enzyme inactivation

Note:

1. For downstream qPCR, the volume of reverse transcription product

added should not exceed 1/10 of the PCR reaction volume. For example, add no more than 5 µl of reverse transcription product to a 50 µl qPCR reaction.

2. **Before the downstream qPCR, place the reverse transcription product on ice. For longer storage, store the reverse transcription product at -30~-15°C.**

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.