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cDNA First Strand Synthesis Kit Instructions

Composition

cDNA First Strand Synthesis Kit Cat. No.	5 Preps 7306005	25 Preps 7306025	100 Preps 7306100
5×gDNA Buffer	15 µl	60 µl	220 µl
RNase-free Water	1.5 ml	1.5 ml	1.5 ml×2
5×RT Buffer	25 µl	110 µl	420 µl
RT Enzyme Mix	15 µl	60 µl	220 µl
RT Primer Mix	25 µl	110 µl	420 µl

Storage

Store at -20℃ with an expiration date of 2 years.

Technical Support

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Introduction

This kit is an efficient, rapid, and genomic DNA decontamination reverse transcription system, with simple operation steps, and can complete synthesis of the first strand of cDNA within half an hour, which is very suitable for real-time PCR and common RT-PCR experiments. This kit contains dsDNase with efficient genomic DNA digestion (dsDNase can specifically digest double-stranded DNA), and gDNA can be digested at 42℃ in 3 min, which effectively avoids the interference of genomic DNA in total RNA, and does not digest primers, probes, RNA, and subsequent synthesis of cDNA, which improves the sensitivity of the experiment. A high-efficiency reverse transcriptase included in this kit, can be used to synthesize the first strand of cDNA in 15 minutes at 42℃. The kit also has the characteristics of high affinity with RNA templates, which can read through RNA templates with high GC content and complex secondary structure.

Equipment And Reagents to Be Supplied by Users

1. A water bath or a PCR instrument.
2. Ice bath.
3. RNase-free PCR tubes.
4. Pipettes and RNase-free tips.
5. Disposable gloves, masks and protective equipment and tissues
6. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml centrifuge tubes)

Cautions

1. The following protocol should be performed on an ice bath to avoid RNA degradation.
2. For RNA templates with very complex secondary structures, it is recommended that the template RNA be incubated at 65℃ for 5 min and then quickly transferred to an ice bath prior to cDNA synthesis.
3. If you need to use Oligo-dT or gene-specific primers alone, please add the primers in the ratio of Oligo-dT Primer 50 pmol/20 µl or reverse gene-specific primers 5 pmol/20 µl and replace the RT Primer Mix with RNase-free Water to 4 µl.

Protocol

The following protocol is applicable to total RNA with a template volume of 50 ng-2 μg, and if the total RNA amount is greater than 2 μg, scale up the reaction system.

1. **Thaw the template RNA, 5× gDNA Buffer, RNase-free Water, 5×RT Buffer, RT Primer Mix, and place on ice quickly after thawing. Invert each solution several times before use and spin down the liquid remaining on the tube wall.**
2. **Prepare the mixture according to the genomic DNA digestion system in Table 1, mix thoroughly, spin down several seconds, and incubate at 42℃ for 3 min. Then place on ice.**

* RNA integrity is important for reverse transcription, and degradation of the RNA template will result in a reduction in cDNA product or inability to amplify long fragments of genes.

Table 1: gDNA digestion system

5×gDNA Buffer	2 μl
Total RNA	n μl (50 ng-2 μg)
RNase-free Water	(8-n)μl

3. **Prepare the mixture according to the reverse transcription reaction system in Table 2.**

* If you want to synthesize multiple tubes of different cDNA at once, add and mix the components of the reverse transcription reaction into a PCR tube in advance, and then divide into the gDNA remove reaction as 10 μl/tube to reduce experimental error.

Table 2: Reverse transcription response system

5×RT Buffer	4 μl
RT Enzyme Mix	2 μl
RT Primer Mix	4 μl

4. **Add the mixture from the reverse transcription reaction to the mixture of the gDNA digestion system, mix well, and spin down the remaining liquid on the tube wall.**
5. **Incubate at 42℃ for 15 min.**
6. **Incubate at 95℃ for 3 min and place on ice, the cDNA can be used for subsequent experiments, or stored at -20℃.**

* Steps 5 and 6 can be done on a PCR instrument.

* If the subsequent experiment is real-time PCR, the amount of cDNA should not exceed 1/10 of the final volume of the PCR system, for example, for a 50 μl PCR system, the amount of cDNA should not exceed 5 μl.

* If the cDNA does not show a standard "S" curve when used as a real-time PCR, it may be that the concentration of cDNA is too high and affects the acquisition of background fluorescence, and it is recommended to dilute the obtained cDNA 2-5 times before performing a real-time PCR experiment.