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All-in-one 1st Strand cDNA Synthesis Kit (gDNA Purge) Instructions

Composition

All-in-one 1st Strand cDNA Synthesis Kit（gDNA Purge） Cat. No.	50 Preps 7316050	100 Preps 7316100
5×All-in-one RT Buffer	210 µl	420 µl
Enzyme Mix	55 µl	110 µl
No RT Control Enzyme Mix	10 µl	20 µl
RNase-free Water	1.5 ml	2 ml

Storage

Store at -20℃ with an expiration date of 1 year.

Technical Support

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Introduction

The All-in-one 1st Strand cDNA Synthesis Kit (gDNA Purge) is an upgraded version of the cDNA 1st Strand Synthesis Kit, which can perform both genomic DNA remove and RNA reverse transcription reactions in the same tube, reducing the risk of sample contamination and RNA degradation caused by complex loading processes. In addition, the kit also provides No RT Control Enzyme Mix, which can be used to configure a control group without reverse transcriptase to determine whether genomic DNA remains in the RNA template.

This kit uses dsDNase to efficiently remove genomic DNA, which is different from conventional DNase I, which can specifically digest double-stranded DNA (dsDNA, hybrid strand of DNA and RNA), and does not digest primers, RNA, and subsequent synthesis of cDNA, which improves the sensitivity of the experiment, dsDNase is thermally sensitive and can be quickly and irreversibly inactivated at high temperatures without affecting subsequent experiments. The kit contains a high-efficiency reverse transcriptase that can withstand a reaction temperature of 55℃ and is suitable for reverse transcription of RNA templates with complex secondary structures. The synthesized 1st-strand cDNA can be used directly in PCR or real-time PCR (including dye and probe methods).

Equipment And Reagents to Be Supplied by Users

1. PCR instrument.
2. Ice bath.
3. RNase-free centrifuge tubes and 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. Reaction preparation should be performed on an ice bath to avoid RNA degradation.
2. RNA integrity is important for reverse transcription, and if the RNA template is degraded, it will result in a reduction in cDNA products or the inability to amplify long fragments of genes, so try to use high-quality RNA templates.
3. 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.

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Protocol

1. **Thaw the template RNA, 5 × All-in-one RT Buffer, RNase-free Water at room temperature (15-25℃) and place on ice immediately after thawing. Invert each solution several times before use, and spin down the liquid remaining on the tube wall.**
2. **Prepare the reaction mixture following the table below.**

5×All-in-one RT Buffer	4 µl
Enzyme Mix	1 µl
Total RNA	n µl (1 of 2 µg)
RNase-free Water	to 20 µl

Gently pipette or flick the wall of the tube until well mixed, and spin down the liquid on the wall of the tube.

* If you want to synthesize multiple tubes of different cDNA at once, you can pre-add the components of the reaction to a single centrifuge tube and mix well, then divide (20-n) µl/tube into the PCR tube and add RNA to simplify the operation and reduce experimental errors(Due to pipette errors, usually the reaction solution of mixed x samples is only enough to aliquot x-1 PCR tubes, it is recommended to add one tube when calculating the number of samples).

3. **Prepare No RT Control Reaction Mix (optional) following the table below.**

No RT Control refers to a negative control reaction without reverse transcriptase to verify the presence of genomic DNA residue in the RNA template.

5×All-in-one RT Buffer	4 µl
No RT Control Enzyme Mix	1 µl
Total RNA	n µl (1 of 2 µg)
RNase-free Water	to 20 µl

Gently pipette or flick the wall of the tube until well mixed, and spin the liquid remaining on the wall of the tube.

4. **Reaction protocol**

Temperature	Time
37℃	5 min
50℃	15 min
95℃	3 min

5. **The resulting cDNA can be used immediately for subsequent experiments or stored at -20℃.**

* If the subsequent experiment is a real-time PCR, the amount of cDNA should not exceed 1/10 of the final volume of the PCR system, e.g., 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.