TOOLS Easy Fast RT Kit

For fast first-strand cDNA synthesis and removal of gDNA

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Introduction

TOOLS Easy Fast RT Kit is designed for fast high-efficiency first-strand cDNA synthesis and is able to rapidly remove genomic DNA (gDNA). The kit contains an RT enzyme, an RNase inhibitor, random primers, an Oligo dT primer, a dNTP mixture, and a reaction buffer. It takes only 15 min (at 42°C) to complete the synthesis of first-strand cDNA using the innovative RT enzyme. This product also has a high affinity for RNA, which enables efficient and sensitive reverse transcription of any template (such as GC-rich templates and templates of complicated secondary structures of RNA), resulting in high yields of cDNA.

Applications

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

Important Notes

1. The protocol is optimized for use with 50 ng to 2 μg of RNA. With >2 μg of RNA, scale up the reaction linearly to the appropriate volume.
2. Perform the experiment on ice to avoid the degradation of RNA.
3. For complicated secondary structures of template RNA, incubate RNA for 5 min at 65°C and then chill on ice immediately before performing reverse transcription.
4. The use of the Gene Specific Primer is not suitable for this kit.
5. Reaction volume can be proportionally scaled up.

Kit Contents

Contents	KRT-BA18 (100 preps)
5×Easy Fast RT mix	400 μl
RNase- Free ddH2O	2x 1 ml

Storage

TOOLS Easy Fast RT Kit can be stored at −20°C for up to 12 months.

Protocol

The total reaction volume of 20 µL is for 50 ng to 2 µg of RNA

1. Thaw the template RNA on ice. Thaw the 5 × Easy Fast RT mix and RNase-Free ddH2O at room temperature and then immediately place them on ice. Vortex and centrifuge briefly to collect residual liquid from the sides of the tubes before use.
2. Perform the following steps on ice. To ensure the exactness of the preparation of the RT reaction, make a master mix and then aliquot for each reaction tube.
3. Prepare the reaction mix on ice according to the specifications listed in Table 1.

Table 1

Solution	Volume
5×Easy Fast RT mix	4µl
Total RNA	50ng-2µg
RNase-Free ddH2O	Up to 20µl

4. Perform the RT reaction according to specifications in Table 2.

Table 2

Temperature	Time
42°C	15 mins
95°C	3 mins

5. The synthesized cDNA can be used for subsequent experiments or stored at −20 °C.

Note: Reverse transcriptase uses template RNA to synthesize first-strand cDNA, and the purity and integrity of template RNA affect the quality of reverse transcription. cDNA yields are low if the template RNA contains RNase. Protein, salt, ethanol, and phenol residues in template RNA can 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 50 µL of qPCR reaction mix, cDNA volume should not exceed 5 µL).