ToolQuant RT Kit

For first-strand cDNA synthesis and two-step RT-PCR

Contents

Introduction

The ToolQuant RT Kit is designed for first-strand cDNA synthesis from a tiny amount of total RNA or mRNA for two-step reverse transcription polymerase chain reaction (RT-PCR). ToolQuant Reverse Transcriptase is a new, unique enzyme and is different from the reverse transcriptases of Moloney murine leukemia virus (MMLV) or avian myeloblastosis virus (AMV). ToolQuant Reverse Transcriptase is a recombinant heterodimeric enzyme expressed in *Escherichia coli*. ToolQuant Reverse Transcriptase has a high affinity for RNA, which enables the efficient and sensitive reverse transcription of any template, leading to high yields of cDNA.

Important Notes

1. Solutions (water and other solutions) should be treated with 0.1% diethyl pyrocarbonate (DEPC) and autoclaved for 15 min to remove any trace of DEPC. For reagents not suitable for autoclaving, prepare the reagents with water in a sterilized container and then filter to obtain the final solutions.
2. Avoid DNA contamination for RNA samples.
3. Repeated freezing and thawing of RNA should be avoided, because this reduces the DNA quantity.
4. All the components in the kit should be stored at −20°C.
5. cDNA synthesized using this kit should be stored at −20°C.

Kit Contents

Contents	TGKRA03 (100 preps)
ToolQuant Reverse Transcriptase	2× 50 μL
Oligo$(dT)_{15}$ (10 μM)	240 μL
Random Octamers (10 μM)	240 μL
10× RT Mix	200 μL
RNase-free ddH_2O	2× 1 mL
Super pure dNTP (2.5 mM each)	240 μL

Storage

The ToolQuant RT Kit can be stored at −20°C for up to 12 months.

Protocol

The protocol is optimized for use with 50 ng to 2 µg of RNA. For >2 µg RNA, scale up the reaction linearly to the appropriate volume.

1. Thaw template RNA on ice. Thaw the primer solutions (not supplied), 10× RT Mix (including RNase and dithiothreitol [DTT]), super pure dNTPs (2.5 mM each), and RNase-free ddH_2O at room temperature (15°C–25°C). Place the template on ice immediately after thawing. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.
2. Prepare a fresh master mix on ice according to Table 1. Mix thoroughly and carefully by vortexing for no more than 5 s. Centrifuge briefly to collect residual liquid from the walls of the tube, and store on ice. Add the RNA template in Step 4.

Note: Set up 10 µL reverse transcription reaction for downstream qPCR.

3. If setting up more than one reverse transcription reaction, distribute the appropriate volume of the master mix into individual reaction tubes. Keep the tubes on ice.

Note: A volume of master mix 10% greater than required for the total number of reverse transcription reactions should be performed.

4. Add the template RNA (50 ng–2 µg) to the individual tubes containing the master mix. Mix thoroughly and carefully by vortexing for no more than 5 s. Centrifuge briefly to collect residual liquid from the walls of the tubes.
5. Incubate for 60 min at 37°C.
6. Add an aliquot of the completed reverse transcription reaction to the PCR mix.

Table 1. Reverse Transcription Reaction Components

Component	Volume/Reaction	Final Concentration
10x RT Buffer	2 µL	1×
Super pure Dntp (2.5 mM each)	2 µL	0.25 mM each dNTP
Oligo-$(dt)_{15}$ or Random (10 µM)*	2 µL	1 µM
ToolQuant Reverse Transcriptase	1 µL	(20 µl reaction system)
RNase-free ddH_2O	× µL	
Template RNA added in the step 5	× µL	
Total reaction volume	20 µL	

*Provided. If gene-specific primers are required in some specific experiments, a final primer concentration ranging from 0.1 to 1.0 µM can be used.