Quantscript RT Kit

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

www.tiangen.com/en

Quantscript RT Kit

Cat. No. 4992783/4992784

Kit Contents

Contents	4992783 (25 rxn)	4992784 (100 rxn)
Quant Reverse Transcriptase	25 μl	2 × 50 μl
Oligo(dT)$_{15}$ (10 μM)	60 μl	240 μl
Random (10 μM)	60 μl	240 μl
10× RT Mix	50 μl	200 μl
RNase-Free ddH_2O	1 ml	2×1 ml
Super Pure dNTPs (2.5 mM each)	60 μl	240 μl
Handbook	1	1

Storage

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

Introduction

Quantscript RT Kit is designed for the first strand cDNA synthesis from tiny amount of total RNA or poly(A)$^+$RNA for high sensitive two-step RT-PCR. Quant Reverse Transcriptase is a new, unique enzyme, different from the reverse transcriptases of Moloney murine leukemia virus (MMLV) or avian myeloblastosis virus (AMV). Quant Reverse Transcriptase is a recombinant enzyme expressed in *E. coli*. Quant Reverse Transcriptase has a high affinity for RNA, which enables efficient and sensitive reverse transcription of any template, leading to high yields of cDNA.

Product Features

This kit includes all relative contents of cDNA first strand synthesis. Quant Reverse Transcriptase in this kit contains high reverse transcriptase activity. This product have good performance with RNA templates with high GC content and complicated secondary structure, have high compatibility toward downstream PCR or real time PCR and could be combined with any thermostable polymerase.

Important Notes Before Starting

1. Solutions used for synthetic reaction of cDNA should be treated with 0.1% DEPC as far as possible and used after autoclaving. For some reagents not suitable for autoclaving, prepare the reagents with the sterilized container and water, and then filter to obtain the final solutions.
2. Avoid genomic DNA contaminations for RNA sample.
3. Repeated freezing and thawing of RNA should be avoided, since this leads to reduced RNA quantity.
4. All the components in the kit should be stored at -30~-15°C.
5. cDNA synthesized by this kit should be stored at -30~-15°C.

Protocol

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

1. Thaw template RNA on ice. Thaw the primer solutions (not supplied), 10×RT Mix (including RNasin and DTT), Super pure dNTPs (2.5 mM each), and RNase-Free ddH_2O at room temperature (15-30°C). Place on ice immediately after thawing. Mix each solution by vortex, and centrifuge briefly to collect residual liquid from the inside walls of the tubes.
2. Prepare a fresh master mix on ice according to Table 1. Mix thoroughly and carefully by vortex. Centrifuge briefly to collect residual liquid from the inside walls of the tube, and store on ice. Add the RNA template in the step 4.

   Note: Set up 10 μl reverse-transcription reaction for downstream Real Time qPCR. All the components can be reduced to 1/2 to adapt for RealUniversal Color PreMix (4992929/4992881/4992904), SuperReal PreMix Plus (4992214/4992215/4992248&4992290/4992291/4992305) and FastFire qPCR PreMix (4992217/4992218/4992249&4992220/49 92221).

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

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

4. Add the template RNA (50 ng-2 μg) to the individual tubes containing the master mix. Mix thoroughly and carefully by vortex for no more than 5 sec. 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 finished RT products to the PCR mix.

Table 1. Reverse-Transcription Reaction Components

Component	Volume/reaction	Final concentration
10x RT Mix	2 μl	1×
Super pure dNTPs (2.5 mM each)	2 μl	0.25 mM each dNTP
Oligo$(dT)_{15}$ or Random (10 μM)*	2 μl	1 μM
Quant Reverse Transcriptase	1 μl	(20 μl reaction system)
RNase-Free ddH_2O	X μl	
Template RNA (add in step 4)	X 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 μM to 1.0 μM can be used.