TIANScript II RT Kit

For complex secondary structures and long first-strand cDNA synthesis

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TIANScript II RT Kit

Cat.No. 4992910/4992911

Kit Contents

Contents	4992910 25 rxn	4992911 100 rxn
TIANScript II RTase (200 U/μl)	25 μl	100 μl
Oligo(dT)$_{15}$ (10 μM)	60 μl	240 μl
Random (10 μM)	60 μl	240 μl
5× TIANScript II RTase Buffer	150 μl	500 μl
RNase-Free ddH_2O	1 ml	2 × 1 ml
Super Pure dNTPs (10 mM each)	30 μl	120 μl
RNasin (40 U/μl)	15 μl	2 × 30 μl
Handbook	1	1

Storage

All the components can be stored at -30~-15°C for up to 12 months.

Introduction

TIANScript II RT Kit supplies all the required reagents for first-strand cDNA synthesis, including the TIANScript II RTase which is modified from M-MLV, with higher affinity to template and lower RNase H activity. Therefore it has the ability to read complex secondary structure of template and reverse transcribe long RNA in the process of first-strand cDNA synthesis. The 5×TIANScript II RTase Buffer in the kit not only guarantees high enzyme activity of new RTase, but also expands the template input range. cDNA synthesized by this kit has high quality and is good for downstream analysis.

Product Highlights

High enzyme activity: High RTase activity, which is compatible for downstream analysis.

Wide range of substrates: Suitable for reverse transcription of all kinds of RNA, especially for RNA which has complex secondary structure.

Long RT fragments: The maximum length of first-strand cDNA the kit can synthesize is up to 12 kb.

Applications

1. Synthesis of first-strand cDNA.
2. Construction of cDNA library.
3. Two step RT-PCR.
4. RACE analysis.

Source of Enzyme

Recombinant *E. coli* which contains improved M-MLV gene.

Unit Definition

1. 1 unit (U) is defined as the amount of enzyme required to catalyze the incorporation of 1 nM of dNTP into acid-insoluble in 10 min at 37°C, using polyA.poly(dT)$_{12\text{-}18}$ as template and primers.

Important Notes Before Starting

1. Solutions that used for cDNA synthesis should be treated with DEPC and autoclaved before use. For 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 contamination in RNA sample.
3. Repeated freezing and thawing of RNA should be avoided. Keep the dissolved RNA on ice.
4. All the components of the kit should be stored at -30~-15°C.
5. When using Random Primers, please note the ratio of the amount of primers and the amount of RNA. It is suggested to use 50 ng of Random Primers for every 5 μg total RNA template. Increase the ratio of Random/RNA will improve the synthesis of short cDNA (~500 bp); and decrease the ratio of Random/RNA will improve the synthesis of long cDNA.
6. For RNA templates rich in secondary structures, it is suggested to heat at 65°C for 5 min before the reaction.
7. If the reverse primer of PCR reaction would be used in reverse transcription, in order to avoid nonspecific amplification led by primer mismatch, the whole transcription process could be done at 50°C.
8. We suggest to terminate the synthesis at 70°C for 15 min in order to avoid cDNA damage.
9. When using the enzymes, please mix enzymes gently to avoid bubbles; please centrifuge carefully to collect the enzyme before pipetting, and please aspirate slowly since enzymes are sticky.

   Note: For long cDNA synthesis, please prepare fresh, integrate and pure RNA template.

Protocol

1. Thaw the template RNA on ice; Thaw the primers, 5× TIANScript II RTase Buffer, Super Pure dNTPs and RNase-Free ddH_2O at room temperature (15-30°C) and then place all the reagents on ice. Mix all the reagents by vortex and briefly centrifuge before use to collect drops from the inside of the tube to the bottom.
2. Setup the reaction in a nuclease-free microcentrifuge tube on ice according to the following table:

Reagent	Volume (μl)
RNA	1 ng-2 μg total RNA or 1 pg-2 ng Poly(A) mRNA
Primer	2 μl Oligo-(dT) (10 μM) or 2 μl Random Primer (10 μM) or 10-15 pmol gene-specific primers
Super Pure dNTPs (10 mM)	1 μl
RNase-Free ddH_2O	Up to 14.5 μl

3. Incubate at 65°C for 5 min and then cool on ice immediately for 2 min.
4. Continue to setup the reaction according to the following table:

Reagent	Volume (μl)
Reaction from Step 3	14.5
5× TIANScript II RTase Buffer	4
RNasin (40 U/μl)	0.5
TIANScript II RTase (200 U/μl)	1
Total Volume	20

5. If using Random Primers, incubate the microcentrifuge tube at 25°C for 10 min. If using Oligo-dT or gene-specific primers, skip this step.
6. Incubate at 42°C for 60 min.
7. Heat the reaction mixture at 85°C for 5 min (or 70°C for 15 min) to terminate the reaction, and then place on ice for downstream experiments or store at -20°C immediately.