TIANScript II M-MLV

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

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TIANScript II M-MLV

Cat. no. 4992999/4993000

Kit Contents

Contents	4992999 25 rxn	4993000 100 rxn
TIANScript II RTase (200 U/μl)	25 μl	100 μl
5×TIANScript II RTase Buffer	150 μl	500 μl
Handbook	1	1

Storage

TIANScript II M-MLV can be stored at –20°C for up to 12 months.

Introduction

TIANScript II RTase is an RNA-dependent DNA polymerase and consists of a single subunit with a molecular weight of 71 kDa. It can be used in cDNA synthesis with RNA or RNA: DNA hybrids as templates. TIANScript II RTase is a modified Moloney's mouse leukemia virus reverse transcriptase (M-MLV), which has higher template affinity and lower RNase H activity, so it has the ability to read through the complex secondary structure template and reverse transcription long cDNA fragments in the synthesis of the first strand of cDNA. The 5 × TIANScript II RTase Buffer equipped in the product has been carefully optimized, which not only ensures the efficient enzyme activity of the new reverse transcriptase, but also widens the range of RNA template addition, so that the reverse transcribed cDNA has better quality for subsequent experimental analysis.

Product Features

High enzyme activity: The TIANScript II RTase has high reverse transcriptase activity and good compatibility in subsequent experiments.

Wide range of substrates: Suitable for all RNA, especially RNA template with complex secondary structure.

Long reverse transcription fragments: Up to 12 kb first strand cDNA can be synthesized.

Application

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

Product Resource

Recombinant *E. coli* strain, with modified Moloney Murine Leukemia Virus Reverse Transcriptase gene.

Unit Definition

One unit is defined as the amount of enzyme that incorporates 1 nM dNTPs into acid-insoluble material within 10 min at 37°C with polyA-poly $(dT)_{12\text{-}18}$ as the template-primer.

Protocol

Synthesis of first-strand cDNA

1. Thaw the template RNA on ice. Thaw primers, 5×TIANScript II RTase Buffer, dNTP mixture and RNase-free ddH_2O at room temperature (15-25°C), and transfer on ice immediately after thawing. Completely mix the solutions by vortex, and centrifuge briefly to collect the liquid on the tube wall.
2. Prepare the reaction mix in a RNase-free tube on ice.

Components	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
dNTPs (10 mM, Each)	1
RNase-free ddH_2O	Up to 14.5

3. Heat at 65°C for 5 min, and place the tube immediately on ice for 2 min.
4. Prepare 20 μl reaction mixture by adding the following components to the mixture above.

Components	Volume (μl)
The above mixture	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 the primer is random primer, incubate the reaction system at 25°C for 10 min. If the primer is oligo dT or gene specific primer, this step can be skipped.
6. Incubate at 42°C for 60 min.
7. Terminate the reaction at 85°C for 5 min (or at 70°C for 15 min). Place the tube on ice for follow experiments or freeze to store.

Notes **Please read these notes before use.**

1. This product does not include dNTP mixture and RNasin (RNase inhibitor), pleas purchase separately.
2. The reagent used in the synthesis of cDNA should be treated with DEPC and used after autoclaving. For some reagents that cannot be sterilized under high pressure, prepare with sterilized instruments, water, etc., and then filter the solution.
3. Avoid genomic DNA pollution of RNA samples.
4. Avoid repeated freezing and thawing of RNA to keep it in the thawed state in the ice bath.
5. Each component in the kit shall be stored at - 20°C.
6. When using random primer, we should pay attention to the relationship between the amount of random primers and the total RNA. Generally, we suggest 50 ng random primers/5 μg total RNA. When the ratio of random primers/RNA is increased, the synthesis of short fragment (~ 500 bp) cDNA will be increased; When the ratio of random primer/RNA is reduced, the synthesis of long cDNA fragments will be increased.
7. If the template RNA is rich in secondary structure, it can be treated at 65°C for 5 min.
8. If PCR downstream primers are used in reverse transcription, in order to prevent nonspecific amplification caused by primer mismatch, reverse transcription can be carried out at 50°C.
9. In order to prevent the destruction of cDNA structure, it is suggested that the enzyme should be inactivated at 70°C for 15 minutes.
10. When using enzymes, mix them gently to avoid foaming. Before pipetting, carefully centrifuge the solution to the bottom of the reaction tube. Due to the high viscosity of the enzyme, it should be aspirate slowly.
11. **Special note: To amplify long fragments, please use freshly extracted RNA with good integrity and high purity.**