ToolScript MMLV RT Kit

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

Contents

Introduction

ToolScript RT Kit is designed for first-strand cDNA synthesis either from tiny amounts of total RNA or poly(A) +RNA for subsequent use in two-step RT-PCR. The kit is then applied in combination with PCR to detect the expression of rare genes, the quantitative expression level of mRNA in a very small number of cells, and the cloning of the specific gene's cDNA.

Important Notes

1. Solutions (such as water) should be treated with 0.1% DEPC and autoclaved for 15 min to remove any trace of DEPC. For reagents that are not suitable to be autoclaved, prepare the reagents using water and the sterilized container, and then filter to obtain the resultant solution.
2. Avoid DNA contamination in the RNA sample.
3. Avoid repeatedly freezing and thawing the RNA.
4. Keep the dissolved RNA on ice.
5. All components in the kit should be stored at −20°C.
6. cDNA synthesized by this kit should be stored at −20°C.

Kit Contents

Contents	TGKRA04 (100 preps)
ToolScript M-MLV (200 U/μL)	100 μL
Oligo(dT)15 (10 μM)	240 μL
Random Octamers (10 μM)	240 μL
5 × first-strand buffer	500 μL
RNase-free ddH_2O	2 × 1 mL
Super pure dNTP (10 mM each)	120 μL
RNasin (40 U/μL)	2 × 30 μL
Handbook	1

Storage

All components should be stored at −20°C. ToolScript M-MLV can be stored at −20°C for up to 12 months.

Protocol

1. Prepare the reverse-transcription reaction mix by combining the following components in a sterile RNase-free 1.5-mL microcentrifuge tube on ice.

No.	Component
1	1–5 μg of total RNA or 50–500 ng of mRNA (recommended RNA input: 1 ug)
2	2 μL of Oligo(dT)15 (10 μM), 2 μL of Random Octamers (10 μM), or 2 pmole of gene-specific primers
3	0.5 μL of super pure dNTP (10 mM each)
4	Add RNase-free ddH2O to bring the total volume to 14.5 μL

2. Heat at 70°C for 5 min and then cool on ice immediately for 2 min. Briefly centrifuge and add the following components.

No.	Component
1	4 μL of 5 × first-strand buffer (including DTT)
2	0.5 μL of RNasin (40 U/μL)

3. Add 1 μL (200 U) of ToolScript M-MLV. Mix gently by pipetting the solution. If using Random Octamers, incubate the microcentrifuge tube at 25°C for 10 min (If using polyA, one unit is defined as the amount of enzyme that incorporates 1 nmol of dNTPs into acid-insoluble material within 10 min at 37°C with polyA/poly (dT)12-18 as the template/primer).
4. Incubate at 42°C for 50 min.
5. Heat the sample at 95°C for 5 min to inactivate ToolScript M-MLV and then immediately place on ice for downstream experiments or store at −20°C. (If RNase H is required, perform step 4. Otherwise, proceed directly to step 5.)
6. Add 1 μL of RNase H (2 U), incubate at 37°C for 20 min to remove RNA, and then heat the sample at 95°C for 5 min to inactivate the RNase H.
7. Dilute the reaction to 50 μL with RNase-free ddH_2O. Take 2–5 μL of the diluted liquid for direct PCR amplification.

PCR Amplification

Take 10% of the reaction mixture (2 μL) of the first-strand cDNA synthesis for PCR. Larger amounts of cDNA synthesis products may not result in highly efficient DNA amplification, and inhibitors presenting in the reverse-transcription products may also inhibit the PCR.

1. Prepare the reaction mixture by adding the following components to a microcentrifuge tube.

Reagents	Volume
10 × PCR Buffer (200 mM Tris-HCl [pH 8.4], 500 mM KCl)	5 μL
50 mM $MgCl_2$	1.5 μL
Super pure dNTP (10 mM)	0.25 μL
Primer 1 (10 μM)	1 μL
Primer 2 (10 μM)	1 μL
Taq DNA Polymerase (5 U/μL)	0.4 μL
cDNA (synthesis reaction mixture)	2 μL
ddH_2O	38.85 μL (up to 50 μL)

For optimal results, the concentration of $MgCl_2$ should be optimized for the individual template/primer combination.

2. Mix gently and overlay the reaction with one or two drops (≈50 μL) of nuclease-free mineral oil to prevent evaporation and condensation. The use of mineral oil is not necessary if the thermo cycler is equipped with a hot lid.
3. Set 15–40 PCR cycles. Annealing and denaturation conditions should be optimized for the individual primer/template combination.