USER GUIDE

ARROW-Script Reverse Transcriptase

Cat. ARP4502050
Qty. 10,000U

Content and Storage

Content	Cat	Product	Qty
	ARP4502050	ARROW M-MLV Reverse Transcriptase	10,000U / vial

Storage **-20** ℃

Description

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT). Uses single-stranded RNA or DNA in the presence of a primer to synthesize a complementary DNA strand. The enzyme is used to synthesize first-strand cDNA up to 6 kb.

M-MLV is an RNA-dependent DNA polymerase and consists of a single subunit with a molecular weight of 71kDa. It can be used in cDNA synthesis with RNA or RNA: DNA hybrids as templates. M-MLV is the preferred reverse transcriptase for long mRNA templates (>6kb), since its RNase H activity is weaker than commonly used reverse transcriptase. It greatly reduces the degradation of RNA templates and therefore increases the productivity.

Application

Synthesis of first-strand cDNA, One-Step RT-PCR, 3' and 5' RACE PCR, prime extension, cDNA library construction, etc.

Unit Definition

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.

Standard Protocol for First-Strand cDNA Synthesis

Synthesis of first-strand cDNA 20 µl reaction system can be used for reverse transcription of 1-5 µg total RNA or 50-500 ng mRNA.

1. Add the following components to a nuclease-free micro-centrifuge tube.
   2 µl……… oligo (dT)12-18 (10 µM), or 2 µl random primers (10 µM)
   or 2 pmol gene-specific primers
   2 µl……… dNTPs (10 mM each)
   1-5 µg……… total RNA, or 50-500 ng mRNA
   Add RNase-free ddH_2O up to 15 µl.

2. Heat at 70 °C for 5 min, and place the tube immediately on ice for 2 min. Centrifuge briefly and then add 4 µl 5X First-Strand Buffer, and 2 µl 100mM DTT .
   Optional Step : If the amount of starting template is less than 50 ng, 0.5-1 µl Rnase Inhibitor (40 U/µl) should be added.

3. Add 1 µl ARROW M-MLV and mix gently by pipetting; when using random primers, incubate the tube at 25°C for 10 min.

4. Incubate at 42 °C for 50 min.

5. Heat the sample to 95°C for 5 min to inactivate enzyme. Cool the sample on ice for downstream experiments or store at -20°C immediately.
   If the RNase H is needed, perform the step 6. Or, proceed to step 7 directly.

6. 6. Add 1 µl RNase H (2 U), incubate at 37°C for 20 min to degrade RNA. Then heat the sample to 95°C for 5 min to inactivate RNase H.

7. Dilute the reaction system to 50 µl in RNase-free ddH_2O. Take 2-5 µl for PCR amplification.

PCR Amplification

Take 10% of the first-strand cDNA synthesis reaction mixture (2 µl) for PCR; increasing amount of cDNA synthesis products not lead to highly efficient DNA amplification and inhibitors presenting in the reverse-transcription products may inhibit the PCR.

1. Prepare reaction mixture by adding the following components to a microcentrifuge tube.
   2.5 µl………10x PCR buffer
   0.5 µl……… dNTPs (10 mM each)
   0.5 µl……… Forward Primer (10 µM)
   0.5 µl……… Reverse Primer (10 µM)
   2 units……… Taq DNA Polymerase (5 U/ µl)
   2.0 µl……… cDNA (synthesis reaction mixture)
   Add DNase-free ddH_2O up to 25 µl

   Note: To obtain the optimal result, the concentration of $MgCl_2$ should be optimized for 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.
   (Mineral oil is not necessary if the thermo cycler has been equipped with hot lid.)

3. Denature at 94°C for 2 min.

4. Set 15-40 PCR cycles. The conditions of annealing and denaturation should be optimized for individual primer and template.

Additional Protocol

RNase Inhibitor (Cat.RB0478, 2,000U, 40 units / µl)
RNase H (Cat.GR0610, 250U, 10 units / µl)