

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 °C

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 \cdot poly (dT)12-18 as the template-primer.

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Standard Protocol for First-Strand cDNA Synthesis

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

 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 ddH₂O up to 15 μl.

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.

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

Incubate at 42 °C for 50 min.

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.

5 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.

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 μ l) 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.

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

2.5 μl......10x PCR buffer

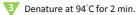
- 0.5 µl..... dNTPs (10 mM each)
- 0.5 μl...... Forward Primer (10 μM)
- 0.5 μl...... Reverse Primer (10 μM)

2 units...... Taq DNA Polymerase (5 U/ μl)

2.0 μ l...... cDNA (synthesis reaction mixture) Add DNase-free ddH₂O up to 25 μ l

Note: To obtain the optimal result, the concentration of ${\rm MgCl}_2$ should be optimized for individual template-primer combination.

Wix 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.)



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)