ARROW-Script Reverse Transcriptase III

Cat. ARP4503050
Qty. 10,000U

Content and Storage

Content	Cat	Product	Qty
	ARP4503050	ARROW M-MLV Reverse TranscriptaseIII	10,000 U / vial

Storage -20 ℃

Supplied 5X First-Strand Buffer :
250 mM TrisHCl, pH 8.3
375 mM KCl
15 mM MgCl 2
50 mM DTT

Description

ARROW-Script Reverse Transcriptase III, is an RNA-dependent DNA polymerase and with reduced RNase H activity and increase thermal stability. The ARROW-Script Reverse Transcriptase III can synthesize 9.5kb products and provide high specificity ,high yields and more full length cDNA.

Application

One unit of activity is the amount of enzyme required to incorporate 1 nmole of dTTP into an acid-insoluble form in 10 minutes at 37°C using polyA-oligo (dT) as template and 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. Mix in the tube: 0.1-5 µg of the total RNA (or 50-500 ng of mRNA) 5 pmole of strand-specific primer (or 250 to 500 ng of oligo -dT or 50-250 ng random primer for each µg of RNA) add water up to 13 or to 14 µl.

2. Incubate the mixture 10 min at 70°C, stand on ice for 1 minute and spin down.

3. Add into the mixture:
   4 µl ..... 5X First-Strand Buffer
   1 µl ..... dNTP mix 10mM
   20-40 units ..... RNase inhibitor (optional)
   1 µl ..... ARROW M-MLV Reverse TranscriptaseIII – 200 units
   Add DNase-free ddH_2O up to 20 µl

   Optional Step : If the amount of starting template is less than 50 ng, 0.5-1 µl RNase Inhibitor (40 units/µl) should be added.

4. Mix well and spin down the mixture, if using random primers incubation at 25°C for 5minutes.

5. Incubate the mixture at 50°C during 30-60 minutes.
   If necessary, can increase to 55 °C for difficult templates or specific gene primer.

6. Heat the mixture 15 min at 70°C to inactivate the RTase.

7. Use the mixture for PCR or for other application.

For your PCR-Reaction you need 1-10 µl of your RT-PCR product.

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)