M-MLV

Retrotranscriptase

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

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M-MLV Retrotranscriptase

Cat. no. EC07

Kit Contents

Contents	EC0701 10000 U	EC0702 20000 U
M-MLV (200 U/μl)	50 μl	100 μl
5× First-Strand Buffer	250 μl	500 μl
Handbook	1	1

Storage

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

Introduction

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 (>5kb), 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 $(dT)_{12\text{-}18}$ as the template-primer.

Protocol

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 $(dT)_{12\text{-}18}$ (10 μM), or 2 μl random primers (10 μM), or 2 pmol gene-specific primers;
   - 1-5 μg total RNA, or 50-500 ng mRNA;
   - 2 μl dNTP mixture (10 mM total, with neutral PH value);
   - 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 5× First-Strand Buffer (with DTT).

   Optional Step: If the amount of starting template is less than 50 ng, 0.5-1 μl RNasin (40 U/μl) should be added.
3. Add 1 μl 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. 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.

Reagents	Volume
10x PCR Buffer (200 mM Tris-HCL (PH 8.4), 500 mM KCl)	5 µl
50 mM $MgCl_2$	1.5 µl
dNTP (2.5 mM each)	1 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	Up to 50 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.