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M-MLV Reverse Transcriptase Instructions

Composition

Cat. No.	8006050	8006200
M-MLV Reverse Transcriptase	50 μl	200 μl
5×RT Buffer	0.5 ml	1 ml
RNase-free Water	1.5 ml	1.5 ml
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Storage

It can be stored for up to two years at -20℃.

Introduction

This product is M-MLV Reverse Transcriptase of Moloney Murine Leukemia Virus expressed by gene recombination technology. In the presence of appropriate primers, it can synthesize its complementary DNA strand using single stranded RNA or DNA as a template.

Wild-type M-MLV Reverse Transcriptase contains RNase H activity and may degrade template RNA in RNA/DNA heterozygotes. The absence of RNase H activity in the mutant M-MLV Reverse Transcriptase enhances its ability to bind to long strand cDNA.

Applications

1. cDNA library construction.
2. RT-PCR reaction and Real Time RT-PCR reaction.
3. Primer extension.
4. RNA sequencing.

Unit Definition

Product concentration of 200 U/μl. Definition of activity unit: Using Poly (A) as template and Oligo (dT) as primer, the amount of enzyme required to catalyze the incorporation of 1 nmol dTTP within 10 min at 37℃ was defined as 1 activity unit (U).

Purity

The purity of the product was more than 90% by Coomassie brilliant blue staining SDS-PAGE. The product was free of endonuclease, exonuclease and RNase contamination.

Equipment and Reagents to Be Supplied by User

1. oligo(dT) $_{12\text{-}18}$ (10 μM) or random primer (10 μM) or 2 pmol gene-specific primer.
2. dNTPs (10 mM each).
3. RNase Inhibitor may be required.
4. RNase-free 1.5 ml microcentrifuge tubes.
5. Pipettes and tips (RNase-free tips with filter).
6. Protective equipment such as disposable latex gloves and paper towels
7. Water bath.
8. A laboratory without RNase, please wear latex gloves and a mask during the whole experiment because RNase is present in saliva and skin.

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Protocol

1. **Add the following regents to a RNase-free microcentrifuge tube:**
   1) **2 µl oligo(dT)$_{12-18}$ (10 µM) or 2 µl random primer (10 µM) or 2 pmol gene-specific primer.**
   2) **0.5-5 µg Total RNA or 50-500 ng mRNA.**

* When total RNA is less than 0.5 µg, it was recommended to add 1 µl RNase Inhibitor (Cat. No. 8008125).

* If the RNA template needs to be incubated at 70℃ for 5 min to destroy the secondary structure, the addition of RNase Inhibitor should NOT be omitted.

   3) **2 µl dNTPs (10 mM each).**
   4) **Add RNase-free Water to 15 µl.**

* If the RNA template is GC-rich or has a complex secondary structure, add the following steps: Incubated at 70℃ for 5 min to destroy the RNA secondary structure, then quickly place on ice to prevent the secondary structure from re-forming, then spin down.

2. **Add the reagent according to the table below:**

The liquid mixture in step 1	**15 µl**
5×RT Buffer	**4 µl**
M-MLV Reverse Transcriptase	**1 µl** *
Total	**20 µl**

* When total RNA is less than 0.5 µg (such as viral RNA reverse transcription), the amount of M-MLV Reverse Transcriptase should be reduced to 0.05-0.5 µl, otherwise it may lead to subsequent PCR amplification to produce non-specific amplification products.

3. **Mix gently, if using random primers as primers, hold at 25°C for 10 min.**
4. **Incubate at 42℃ for 60 min.**
5. **Incubate at 95℃ for 5 min, then cool on ice or store below -20℃ for later use.**
6. **Dilute to 50 µl with RNase-free Water and take 2-5 µl for PCR amplification.**