M-MLV(H-) Reverse Transcriptase

Cat# EP2001 – 10000 U

Storage at -20 ℃

INTRODUCTION

The wildtype Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase has the following activities: RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, and RNase H. The M-MLV (H-) Reverse Transcriptase is a single-site mutant of M-MLV which contains no RNase H activity. Compared with M-MLV mutants obtained via deletion of the RNase H domian, this product, which retains a complete protein structure and polymerase activities, can be used for the synthesis of longer cDNA or the preparation of cDNA library.

CONTENTS

No	Component	**EP2001 – 10000 U**
BA	5X RT Buffer	500 µl
BB	M-MLV (H-) Reverse Transcriptase (200 U/µl)	50 µl

UNIT DEFINITION

One unit (U) is defined as the amount of enzyme that incorporates 1 nmol of dTTPs into acid-insoluble products in 10 min at 37℃ with Poly(rA)-Oligo (dT) as the template / primer.

PROTOCOL

1. Mix the following components in a RNase-free tube and mix gently	
M-MLV (H-) Reverse Transcriptase (200U/µl)	1 µl
5X RT Reaction Buffer	4 µl
10 mM dNTP Mix	1 µl
Oligo $(dT)^{18}$ (50 µM)	1 µl
or Random Hexamer(50 ng/µl)	
or Gene specific primers (2 µM)	
Murine RNase Inhibitor (40U/µl)	1 µl
Template RNA	Total RNA 100 pg-5ug
	Poly A+ RNA 10 pg-500 ng
RNase-free ddH_2O	To 20 µl

2. Programs for the 1st-strand cDNA synthesis:

a. For oligo $(dT)^{18}$

42℃	45 min*
70℃	15 min

b. For Random Hexamers

25℃	10 min
42℃	45 min*
70℃	15 min

c. For Gene Specific Primers

42℃	45 min*
70℃	15 min

*Can be optimized between 30 min and 60 min. Longer time is helpful to obtain longer cDNA (> 5 kb).

3. Incubate at 70℃ for 15 min to inactivate the reverse transcriptase. The cDNA can be used for PCR or be stored at -20℃ immediately. For PCR, it is recommended that the volume of cDNA ≤ 1/10 of total PCR reaction system volume.

PRODUCT USE LIMITATION

These products are intended for research use only.