M-MuLV Reverse Transcriptase

MMLV possesses RNA and DNA depended polymerase activity and weak RNase H activity.

Product Information

Catalog #:	FZ0306, 10 000 U
Name:	**M-MuLV Reverse Transcriptase** M-MLV with 5×Buffer （with DTT）
Concentration:	200 units/μl
Unit definition:	One unit is defined as the required enzyme incorporate 1 nm dNTP into a polynucleotide fraction in 10 min at 37℃, taking polyA－ poly(dT)12-18 as template-primer.
Source:	Recombination of E.coli containing Moloney murine leukemia virus reverse transcriptase gene from clone of Moloney murine .
Storage:	-20°C

Introduction

Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase is a RNA-dependent DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using either single-stranded RNA or DNA template.

Application：Synthesis of the first chain cDNA, cDNA Library construction, one-step RT-PCR, primer extention, 3′ and 5′RACE

Directions for use

Handling and Storage

Store the RT-reaction by -20°C.

Protocol

1. add the next reaction mixture to ice bath tube :
1) template RNA
total RNA 0.1-5μg
or total poly(A)+mRNA 0.1-0.5μg
or unique RNA 0.01pg-0.5μg
2) primer
Oligo(dT)18 (0.5μg/μl) 1μl
Or stochastic primer（0.2μg/μl） 1μl
Or sequence especially primer 20pmol
3) RNase-free ddh2o : constant volume to 11μl
2. Gently mix and water bath for 5 min in 70℃ and chill on ice.
3. Put the tube into ice and add the next composition :

FT-U5412

5×Reaction Buffer 4µl
RNase Inhibitor (40U/µl) 0.5µl
dNTP Mix(10mmol/L) 2µl
add water to 19ul ,gently mix and then water bath for 5 min in 37℃ ; or for 5 min in 25℃ for random primer

4. Spin down for a few seconds. Add 1µl M-MLV RT（200U/µl）
5. Incubate at 42℃ for 60min(if use a random primer,first incubate for 10min in 25℃
6. Inactivate at 70℃ for 10min.

PCR Reaction

1. Transfer 10% volume of first reaction solution (2 µl) to a proper PCR tube .
note: the first reaction solution can be directly used as PCR template without purification ,the dosage is about 1-5µl. if excessively used ,the salt and Random primers in first reaction solution will restrain the activity of DNA polymerase .if purification needed ,it can follow the next :after reaction end of cDNA synthesis (step 6) , add RNase A in reaction system , 10 min in 37℃ ,use DP1501 recover cDNA .
2. add next solution by order .

5µl 10X PCR Buffer
1µl 10mM dNTP mix
1µl 10µM Primer #1 (customer supplied)
1µl 10µM Primer #2 (p customer supplied)
xµl H20 (total reaction volume:49µl)
1µl Taq DNA polymerase

3. Mix thoroughly and add 50µl mineral oil to the surface of liquid.
4. Amplified reaction : according to annealing temperature or gene copy number or technical parameter of Taq DNA polymerase , setting amplified condition , specify reference to specification of DNA polymerase ,the usually cycle number is 30-35
5 Detect the product in agarose containing EB.

Related products

- dNTP Mix, 100mM, pH7, 127823, 40 µmol
- UptiTherm DNA Polymerase, S53921, 1000 U
- DTT, 054725, 5 g
- Ribonuclease A, 918420, 250 mg

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06