Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

MMLV Reverse Transcriptase (RNase H-)

Product Number: RT01

Storage condition

-20°C

Component

MMLV (RNase H-) (200U/uL)
5×RT Buffer (with DTT)

Description

MMLV Reverse Transcriptase isolated from E. coli carrying rat leukemia virus pol gene consists of a single peptide, molecule weight 71kd. MMLV Reverse Transcriptase, RNase H Minus is an RNA-dependent DNA polymerase with no detectable RNase H activity. A point mutation in the RNase H domain increases the thermostability of the enzyme and support greater cDNA yield of full-length transcripts than wild type M-MLV Reverse Transcriptase.

Features

Weak RNase H activity

High cDNA yield

Application

Synthesis of the first chain cDNA Library construction one-step RT-PCR primer extension 3′ and 5′ RACE.

Source

Recombination of E. coli containing Moloney murine leukemia virus reverse transcriptase gene from clone of Moloney murine.

Unit definition

Using Poly (A) as the template and oligo (dT) as the primer, the enzyme required to catalyze the incorporation of 1 nmol of dTTP within 10 minutes at 37°C is defined as one active unit (U).

Quality control

1. Absence of Endonuclease
   1.1. 1μg of Lambda DNA is incubated with 200 units of MMLV Reverse Transcriptase, RNase H Minus, 1×RT Reaction Buffer for 16 hour at 37°C. Following incubation, Lambda DNA is visualized as intact on an ethidium bromide-stained agarose gel to verify the absence of visible Endonuclease.
2. Absence of Nickase
   2.1. 1μg of Type I supercoiled pBR322 is incubated with 200 units of MMLV Reverse Transcriptase, RNase H Minus, 1×RT Reaction Buffer for 16 hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.
3. Absence of Exonuclease
   3.1. 1μg of Lambda DNA / Hind III Markers is incubated with 200 units of MMLV Reverse Transcriptase, RNase H Minus, 1×RT Reaction Buffer for 16 hour at 37°C. Following incubation, Lambda DNA / Hind III Markers is separated by 1% agarose gel and stained with ethidium bromide. Markers remain as intact bands without smearing.
4. Absence of RNase
   4.1. 1ug of RNA is incubated with 200 units of MMLV Reverse Transcriptase, RNase H Minus, 1×RT Reaction Buffer for 4

hour at 37°C. Following incubation, the RNA is visualized as intact band on an ethidium bromide-stained agarose gel to verify the absence of visible RNase.

5. Function Assay

5.1. First-Strand cDNA Synthesis 200 units of enzyme incubated with 2.5ug of 8.3kb RNA for 1 hours at 42°C must synthesize ≥65% cDNA. ≥45% of the cDNA must be >6kb when analyzed by agarose gel electrophoresis.

6. Physical Purity

6.1. The purity is ≥95% as judged by SDS-polyacrylamide gel with Coomassie blue staining.

Protocol

1. 1. add the next reaction mixture to ice bath tube:

1.1. template RNA

total RNA 0.1-5μg or total poly(A)+mRNA 0.1-0.5μg or unique RNA 0.01pg-0.5μg.

1.2. primer

Oligo(dT)18 (0.5μg/μl) 1μl or stochastic primer (0.2μg/μl) 1μl or sequence especially primer.

1.3. 20pmol RNase-free ddH_2O

constant volume to 11μl.

2. Gently mix and water bath for 5 min in 70°C and chill on ice.

3. Put the tube into ice and add the next composition:

3.1. 5×Reaction Buffer 4μl

3.2. RNase Inhibitor (40U/μl) 0.5μl

3.3. dNTP Mix(10mmol/L) 2μl

3.4. add water to 19ul, gently mix and then water bath for 5 min in 37°C, or for 5 min in 25°C for random primer.

3.5. Spin down for a few seconds. Add 1μl MMLV RT (200U/μl).

3.6. Incubate at 42°C for 60min. if use a random primer, first incubate for 10min in 25°C.

3.7. Inactivate at 70°C for 10min.

PCR Reaction

1. Transfer 10% volume of first reaction solution (2μl) 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.

add RNase A in reaction system, 10 min in 37°C, use DP1501 recover cDNA.

2. Add next solution by order.

5μl 10×PCR Buffer

1μl 10mM dNTP mix

1μl 10μM Primer #1 (customer supplied)

1μl 10μM Primer #2 (p customer supplied)

×μl H_2O (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/DNA safe stain.